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THE STRUCTURE OF THE SALIVARY GLAND CHROMOSOMES AND ITS MEANING

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THE most recent review of the structure of the salivary gland chromosomes presented by Metz (1941) at a symposium in Cold Spring Harbor sets out with the statement that nobody knows at present how the salivary gland chromosomes are constructed. We claim to have acquired this knowledge while those words were spoken and printed. Metz has reported the history and the pertinent facts sufficiently to relieve us from reviewing previous work. Only the following points may be presented by way of introduction: Soon after the discovery of the arrangement (Heitz and Bauer) and the genetic importance (Painter) of the salivary gland chromosomes of Diptera, both Koltzoff and Bridges, obviously under the influence of Jacoby's work on intranuclear division, proposed the idea that the salivary chromosomes are bundles of chromonemata with the chromomeres arranged into the disks, derived by intranuclear division of the original chromosomes to 8, 16, 32 . . . up to thousands. This view was generally accepted and there is probably no text and no lecture course (including those of the senior author) in which this interpretation was not taken as a fact. Looking back, it is rather surprising that all of us, with the exception of Metz, accepted this theory in one or the other specific form, in view of the complete lack of evidence and of serious logical obstacles. These latter have been ably assembled and reviewed in Metz's recent paper

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(see p. 31 *ff*). The factual demonstration rested exclusively upon some pictures obtained in stretched and shrunken chromosomes which show irregular longitudinal strands between the disks. (Bauer a.o. see review in Metz.) Believing that chromonemata, if present as a bundle, ought to become visible, just as a leptotene chromonema is actually visible and not a theoretical concept, we tried in this laboratory to stain them with the methods which reveal the finest fibrillar structures in protozoa and to make them visible in cross sections. The result was completely negative. There is no need to discuss the idea that these chromonemata are submicroscopic, as assumed by some (see Metz). Submicroscopic structures are beyond the range of the cytologist and the term chromonema applies to a clearly visible thing in other chromosomes. Metz, who, alone it seems, realized the shortcomings of the polytene chromosome view, nevertheless accepted the idea of internal multiplication of chromonemata but relegated it to the ultramicroscopic field. Otherwise he tried to explain the structure on the basis of Bütschli's alveolar theory which originally had tried to interpret all visible fibrillar structures as the optical sections of rows of alveolar walls. Metz has again presented his views clearly and compared them with the other view-points. Many of his factual observations can be accounted for simply by our new insight into the structure.

A study of the development of the gland cells might be expected to furnish a decision. It is remarkable that in such studies really clear and convincing figures have led only to a four-strand structure of the chromosomes (Frolova, 1937; Painter and Griffen, 1937; Kaufmann, 1931; Metz, see 1941). The assumed subsequent fission into 8, 16, etc., strands can hardly be considered as demonstrated convincingly. We wonder if it would have been claimed at all if it had not been expected. In nuclei where such a multiple division really occurs, *e.g.*, ovarial nurse cells, a loose bundle of metaphaselike strands is visible, very different from the salivary chromosome structure

(see Painter and Reindorp, 1939; Schultz, 1941). In the discussion following Metz's review (*ib.*, p. 39) Kaufmann reminds us of his observation that in larval nuclei he has seen four strands, stating, "In such cases the halves of each of the homologues are often twisted as though relational coiling existed at that time." (See, also, Kaufmann, 1931; Koller, 1935.) This is in agreement with Frolova's statement (1937) that in such developing nuclei the homologous chromosomes are coiled in opposite directions, whereas the chromatids of each are coiled in the same direction. It is difficult to understand what Koller (1935) claims to be relic and relational coiling, seen in chromosomes which already were supposed to be bundles of chromonemata. These observations if properly interpreted are indeed what is to be expected on the basis of our results, which show that the finished salivary chromosome is still a simple tetrad with special features of coiling; (which, however, has *nothing* to do with the crude coiling seen by the authors just quoted).

What we consider to be the solution of the major part of the problem was first envisaged when salivary chromosomes were treated with alkali, originally for purely physico-chemical purposes (a study of double refraction in which Dr. Calvin, of the Department of Chemistry, was interested). It turned out that this treatment produced definite morphological changes, among them the appearance of the so-called lampbrush chromosome (Calvin, Kodani and Goldschmidt, 1940). A careful comparison showed that the lampbrush hairs surrounding the four chromonemata upon which some chromatin was precipitated were derived from the disks of the normal salivary chromosomes. From this it was concluded that the normal chromosome is a tetrad with four chromonemata and that the disks (bands) have as their structural basis a radial arrangement of these hairs, which, however, are not visible before treatment because of their incrustation with chromatin. Certain features (seen in slides treated with alcohol) made us assume that the hairs themselves were loops of secondary fibrils, somehow derived from

the original chromonemata. Painter (1941) has repeated these experiments but gives a different interpretation to the visible effects. He thinks that many chromonemata are present and that the lampbrush hairs are the product of the matrix between the disks. We shall refrain from a detailed discussion of this divergence of opinion (only one such comparison with former work will be taken up below) because meanwhile the junior author has developed a new method of attack which permits the following of the entire sequence of events and, in addition, produces some decisive stages which were formerly unknown. These, in our opinion, make clear the actual structure of these chromosomes and permit the relating of it to that of ordinary chromosomes. No doubt is left any more that the lampbrush hair is derived from the disks, but the idea of secondary fibrils turned out to be wrong. The data have just been published by Kodani (1942).[1] This paper intends to draw some of the conclusions with the addition of a few more facts.

These are the main facts: Alkali treatment shortens the chromosomes by contraction between the disks. Part of the chromatin contained in the disk migrates toward the center of the chromosome, is precipitated on the chromonemata and makes them visible. The disks appear now as a star or aster of hairlike structures (as seen from the pole), attached to the central chromonemata and containing nucleic acid diffusely or in granules; the hairs are always in pairs longitudinally, *i.e.*, one behind the other, a condition which will be explained at once. In heterochromatic parts within the chromosomes the hairs are quickly dissolved, chromatin and skeleton alike, but the chromonemata remain. This is the structure of the lampbrush chromosome which can be reliably obtained by proper treatment. The thermal increment of the process has been measured in thousands of nuclei and certain chemical conclusions were derived by Calvin (Calvin

[1] Unfortunately, the microphotographs of this paper had to be reduced so much that many details are no longer visible. The originals show everything claimed in the text.

and Kodani, 1941), which will be discussed below. How this structure relates to that of the normal chromosome can not be decided in detail on the basis of the facts reported thus far, except that a normal disk must contain the four central chromonemata and radially arranged structures containing chromatin, and in addition another type of chromatin (or more of it, bound differently) in between the radial hairs. The real relation became visible when treatment with urea was introduced (it was actually suggested by a remark in a lecture by Stanley on virus chain molecules). This, if properly combined with alkali (see Kodani for exact procedure), leaves the chromosome *externally* as long as it was, whereas the simple alkali treatment contracts it. But it has actually increased immensely in length by the appearance, within the membrane of the chromosome, of a spiral formerly not visible in each of the synapsed chromosomes. The coils of the spiral are made up of nothing but the four chromonemata from the center of the disks, where they must have been completely contracted (by molecular folding), which have been uncoiled by the treatment. All along the length of these coiled chromonemata bulblike chromatic appendages are attached at equal distances and arranged in pairs within each of the homologous chromosomes. When the coils are artificially stretched the four chromonemata become visible with the bulbs attached to each but pairwise matched in sister chromatids. By further alkali treatment the coils contract again and the bulbs are transformed into lampbrush hairs. Both bulbs and hairs can be shown by protease and nuclease treatment and Feulgen stain to contain a protein skeleton to which is attached nucleic acid. There can be no doubt that the normal disk contains in its center four chromonemata either closely coiled or folded molecularly, to which are attached radially the penultimate chromomeres, clublike in shape (the bulbs), consisting of a proteinic basis covered or incrusted with chromatin. More chromatin distributed between the clubs or bulbs makes the disk solid

and structureless. In the coiled stage the interdiskal parts are not visible. Obviously, they have been transformed somehow into the same four chromonemata without bulbs. (The heterochromatin is again dissolved and in heterochromatic sections—see Kodani, 1942—no bulbs are visible. Regarding the chromocenter see below.) The normal condition between the disks becomes clear when the untreated chromosome is carefully shrunk, a little stretched, and stained with hematoxylin. Between the disks of the flattened chromosome appear four tube-like bands, more or less broad, according to shrinkage, and more or less stretched. This shows that in the normal chromosome between the disks the four chromonemata are so swollen (by hydration or by intussusception of "matrix," whatever that is (see below)), that they fill the entire space and can not be seen or stained. When shrunk they separate and accept stain. The salivary chromosome is therefore a tetrad of four chromonemata, just as is the case in the development of the gland. To each chromonema are attached at what seems to be equal distances the real penultimate chromomeres. Within each disk the chromonemata form a coil or are compactly folded up and therefore the five or so penultimate chromomeres, arranged pairwise, radiate from the central chromonema in each dyad. There are thus about 10 of these penultimate chromomeres in the cross section of the chromosome tetrad. Between the disks there are no chromomeres and the chromonemata are swollen so as to fill the entire cross section of the tetrad. (All the details are found in Kodani's paper, 1942.)

It would be easy to take up one after the other every single morphological structure described by former authors and to show that it expresses different aspects of staining, solution, shrinkage, or swelling of these constituent parts. We do not think this is necessary on a large scale, as a comparative reading of Metz's review and Kodani's paper will make every point obvious. Instead, we propose to apply the facts to a discussion of the im-

plications contained in the new insight into the structure of the salivary gland chromosomes.

Before beginning that discussion, there is one important point which should be dwelt upon, because it was omitted unintentionally in Kodani's recent paper. Bauer, Painter and others put much stress upon a certain condition of the chromosome which is regarded as the real proof of the polytene structure, *i.e.*, longitudinal strands which become visible and stainable between the disks, fewer in Drosophila and more in Chironomus, when the chromosomes are stretched and fixed. When the chromosome is broken, they stick out of the broken end like the straws of a broom. They seem to flow characteristically out of the granules making up the disks and tend to unite in a net, a condition which Metz interprets as a vacuolar structure. There is no doubt that this condition exists and we can produce it at will by treatment with acid alcohol and much stretching, the same method which produces the four strands if applied cautiously. All the strands thus produced are found more or less in one plane because of pressure and stretching. We think that this condition is an artifact produced in the following way. Acid alcohol will dissolve nucleic acid and produce a lengthening of the penultimate chromomeres into the lampbrush hairs. When a lampbrush condition is produced and the membrane has disappeared, the lampbrush hairs tend to flow out like pseudopodia. This is not an exclusive character of the salivary lampbrush stage, but is also found in the vertebrate oocyte where, towards the end of the lampbrush stage, long processes radiate from the chromonemata and fill almost the entire nucleus. [This is also copied in the oocyte stage of the salivary nucleus pictured in Kodani's (1942) figure 1B.] The secondary chromomeres of diplotene and pachytene chromosomes in Orthoptera also tend to form such pseudopodialike radiations, as every cytologist knows. It seems that such a process is involved when salivary chromosomes are treated with acid alcohol. Pseudopodia

or hairlike processes are pushed from the chromatic bulbs (= granules) of the disks; nucleic acid is being dissolved in them and makes them stainable with carmine. The presence of the membrane and the stretching prevents the normal radial arrangement of these processes and they grow into the interstices between the shrunken four chromonemata between the disks. Attaching themselves to the chromonemata and to each other, they produce that typical stretched network, which in some cases might actually be alveolar, as Metz holds. At broken ends this structure becomes rather clear. It is intended to find a method of staining the pseudopodial network differentially from the four chromonemata. But one thing seems sure, that these strands, produced only in the case of acid alcohol treatment, have nothing to do with true chromonemata, though the latter can be made visible by the same method before the "pseudopodia" have been produced.

Turning now to our discussion, the first problem is to relate the structure found in the salivary gland chromosome to the structure of the ordinary chromosome. There seems to be no doubt that the structure is very little different from that of an ordinary tetrad of large chromosomes, say Tradescantia or Trillium. Let us imagine we could take such a tetrad and uncoil it so that the chromonemata are stretched to make visible what normally is called a chromomere. Let us assume that within this so-called chromomere are one or more finer coils of the chromonema with a number of perultimate chromomeres attached to it radially. Let these grow out so as to make a disk and let the chromonemata between these chromomeres swell to the same diameter as the disk, and we shall have produced to all purposes the salivary chromosome. Whereas there are many times more disks visible in a salivary chromosome than leptotene chromomeres, the visible chromomeres of leptotene to pachytene must correspond to whole groups of disks (we shall return to this point below). If we call the bulbs (and lampbrush hairs) the real ultimate or perultimate chromomeres, it is clear that Belling's (1928) ultimate chromomeres are large

compounds of the perultimate ones. Belling estimated the number of all his ultimate chromomeres in a nucleus of Lilium at about 2,200. In the X-chromosome of *Drosophila melanogaster* alone about 1,000 bands are known. This could mean about 5,000 perultimate chromomeres, assuming that the same number as found in the euchromatin is present within the heterochromatic sections. Lampbrush hair seems to be identical in both parts (before it is dissolved in the heterochromatic parts) and the real difference rests within the nucleic acid. (It is a question whether a lampbrush hair or bulb without nucleic acid might still be called a chromomere, or moreover, chromomere-skeleton.)

There is a distance of about 1.3 μ between the bulbs in the stretched condition, so the length of the completely stretched chromonema would be about 6 mm. But there is one serious error in this calculation: Many of the double bands obtained by much stretching might in fact be the separated pairwise asters of penultimate chromomeres in each of the two chromatids. Further, some of the faint bands and the heterochromatic bands may possibly contain fewer chromomeres and a shorter chromonema in between, which would reduce the maximum length considerably, though it would still stretch into the macroscopic field. The length of the completely stretched X-chromosome is ca. 400 μ, with the stretching mostly between the disks and no uncoiling of the disks. By shrinking the interdiskal space with alkali and possibly also flattening the disks (lampbrush stage), the chromosome may be shortened to about 35 μ, and by prolonged treatment it may shrink further to 13 μ (see Kodani, 1942). In this condition it looks almost like an ordinary chromosome. The difference between this and the length of the metaphase chromosome (2–3 μ) must therefore be bridged by a secondary crude coiling. If we reverse this process mentally, we would get the development of a salivary gland chromosome from a metaphase chromosome (after synapsing).

Let us take up a few details. There is first the mem-

brane, or pellicle. Painter formerly assumed the presence of a pellicle in the salivary chromosome, but in his last paper gave up this idea. The problem is important for the proper interpretation of certain structures which have been described. There is no doubt that the chromosomes treated with alkali and urea have a membrane which is being dissolved by the treatment (see Kodani, 1942). This membrane resists digestion with trypsin longer than other structures, and may be obtained as an empty collapsing sac. In many cases even a fine alveolar structure, sometimes with oblique stripes, can be seen. It is of course possible that this membrane is an artifact. In any case it must be a colloidal membrane which is easily liquefied if the two chromosomes which it surrounds separate. (It is also possible that what appears to be separation is non-concrescence prior to the formation of the membrane.) We believe, however, that the membrane is actually present; the experiments of Buck (1942) in attempting to pull the synapsed chromosomes apart, and the stretching experiments of the same author, are in favor of the presence of a membrane. When the chromosomes are stretched, an alveolar structure appears which transforms into oblique striations across the whole. We do not doubt that this observation of Buck's relates to the same structure of the membrane which we saw in treated chromosomes. The same striation was first seen by Koltzoff, who realized its superficial location but took it as an indication of a cylinder of chromonemata. We do not doubt, further, that such striations of the membrane have frequently been mistaken for chromonemata. A part at least of Metz's alveolar structure might possibly be a membrane structure. This membrane might be a special feature of the salivary chromosomes in connection with their function in secretion: Metz reports swelling and collapsing of the chromosomes *in vivo* and we saw the escape of fluid through the membrane under treatment. The membrane thus may serve as an osmotic membrane for the regulation of the exchange of materials connected with the function of the

giant chromosomes in secretion. It might be added that the junior author noticed frequently in one of the stages which shows the membrane especially well (see fig. 1e in Kodani, 1942) that the membrane of the two arms of the second and the third chromosomes are connected by a string through the region of the (dissolved) chromocenter. Painter and Griffen (1937) figured such a connection as an invisible but postulated structure. Here it is visible, but it can not be decided whether it belongs to the membrane, the chromonema, or both.

The next point for discussion is the so-called lampbrush structure of the salivary chromosomes treated with alkali. The term was chosen because of the great resemblance of this stage to the classical lampbrush-chromosome in the growing nucleus of the vertebrate oocyte (Rückert, Carnoy et Lebrun, Maréchal). In some stages (not in all, see, *e.g.,* Maréchal's figures) these unusually long chromosomes resemble closely both in structure and in order of magnitude the salivary chromosomes in the respective stage. The problem is whether this is only a superficial similarity or an actual identity of structure. A careful comparison reveals what we believe to be identity, except for the chromatin. A vertebrate lampbrush chromosome is actually completely free of nuclein and therefore is Feulgen-negative (Koltzoff, 1938). It consists of a chromonema to which are attached excrescences which in some stages are thread- or disklike and in other stages are fine loops. In a cross section these may, at least in some stages, be arranged like a disk of petals around the central chromonema. They are firmly attached to the chromonema, as the micromanipulation experiments of Duryee (1937) show. According to Koltzoff (1938), these loops and hairs are reproductions of the chromonema. In the salivary lampbrush stage the arrangement of the hairs around the chromonema is a very similar one. But the individual hairs contain chromatin, either diffusely or in granules. In the bulb stage which precedes the lampbrush stage the hair is replaced by a club-like structure which appears entirely chromatic; but by

digestion with nuclease all the nuclein can be removed without destruction of the structure; this applies also to the hair stage. The hairs and bulbs therefore are proteinic (digestible with trypsin), just like the hairs and loops of the lampbrush chromosomes of oocytes. In our first communication we assumed that the protein part of the hair was actually a pointed loop. We do not feel so sure about this now, though the possibility can not be denied. Thus it is clear that the skeleton of the lampbrush chromosome produced experimentally is identical with that of the unstretched oocyte chromosome: the latter is, at the stage in which an aster of loops surrounds the axial chromonema, identical with the salivary chromosome after alkali treatment and removal of the nuclein (also the membrane). In the completely stretched salivary chromosome after the uncoiling of the disks, the bulbs (homologous to the loops of the lampbrush oocyte chromosomes plus nuclein) are attached singly to one chromonema, just as the corresponding loops in the oocyte chromosome are (Koltzoff, Duryee). In this condition they are seen in the slides to break off easily and the point of their former attachment to the chromonema appears as a little knob.[2] Finally, the homology of hair and loops becomes clear in the heterochromatic parts of the treated salivary chromosome (see Kodani, 1942). Here the lampbrush structure is first visible as very pale hair even when the heterochromatin is already dissolved, but the hairs afterwards disappear; whether by solution or only optically is not completely clear.

One of the remarkable features of the coiled stage is the appearance of the free ends of the chromosomes. Here the last coil of bulbs consists of much larger bulbs, which may be a natural condition or due to a differential swelling. In addition, the number of bulbs in this last coil (= uncoiled disk) is smaller than otherwise. In Kodani's paper (1942) this group of endwise bulbs has been likened to the telomeres of Muller and others. They must in addition be chemically or physically different

[2] Not mentioned in Kodani (1942), but easily visible.

because the nucleic acid of these end bulbs is dissolved much more slowly by acid-alcohol than that of the other bulbs. Recently Hinton and Atwood (1942) have studied the frequent occurrence of terminal adhesions of the free ends of salivary chromosomes. We, too, have seen this phenomenon frequently and noticed in addition the apparent strength of the cohesion which leads to a breaking off of tips which are attached to another chromosome in chromosome smear preparations. The morphological and chemical differences of the circle of perultimate chromomeres at the tip (telomeres) may be responsible for this, as well as for the affinity of these points between different chromosomes. But they are not heterochromatic, as their solubility shows.

This leads to a discussion of the chemical differences between the different structures revealed in these experiments. There is the proteinic skeleton of the chromonema and its side branches, the skeleton of the hair and bulbs which make up the disks. It is completely digested by trypsin (Caspersson, Mazia, Kodani). But it must somehow be rhythmically different within the disks and between them. In the untreated chromosome the chromonema within the disk is obviously folded (molecularly?) into a sphere or cylinder, but it can be uncoiled under the influence of urea-alkali. Between the disks the chromonema is inflated, which must mean that some kind of hydration or stuffing with some protein (matrix) has occurred. Alkali removes the "matrix" and the chromonema collapses from a tube to a fine thread. These facts must be correlated in some way with the chemical work of Mazia and coll. (1941) and of Caspersson. Mazia finds that digestion with pepsin shortens the chromosome by the collapse of the interdiskal parts without destroying the continuity of the chromosome, *i.e.*, the same effect as is produced by alkali. He assumes therefore the presence of a skeleton protein and a matrix protein between the disks. The latter would be, according to Caspersson (1940) a globulin, the skeleton protein a histone. Mazia (1942) in addition finds an action of protaminase which

does not attack the skeleton but removes the nucleic acid. From this he concludes (still with caution) that a protamine might form the link between the skeleton and the nucleic acid. Applications of this method to the bulb stage may furnish the answer. If this hypothesis is applied to the visible structure the space between the disks would have to be occupied by a configuration of a little histone as a skeleton with much globulin in such a way that the "inflation" in normal condition and the shrinkage with restoration of the skeleton thread under the influence of alkali or pepsin is possible. Another point is that the "inflated" structure does not have attached nucleic acid, whereas Feulgen positive thymonuclein is deposited upon the skeleton after shrinkage (see Kodani, 1942). It may be that such facts are indicative of the third protamine like protein, which Caspersson suspects of forming the actual skeleton, and which Mazia suspects of attaching the nucleic acid to the histone. This would mean either that the invisible protamine chain which forms the skeleton is surrounded by the hydrated matrix of globulinlike protein which prevents the attachment of nucleic acid, or that the protamine which serves the attaching function is absent. After shrinkage, *i.e.*, removal of the globulin, the skeleton chain remains just as within the disks and is able to attach whatever free nucleic acid is available either directly or, if Mazia is right, via protamine. If Mazia's chemical picture were correct, we might apply it to the morphological picture in the following way: The visible chromonema would be based upon a histonelike chain. To these would be attached in side-chain fashion at distances of about 13,000 Å protamine hairs, which would correspond to the skeleton of the lampbrush hair and bulbs. To these in turn the nucleotides would be attached, the chromatin of the bulbs. If such a thing as a corpuscular gene actually exists, the bulb or perultimate chromomere would be first entitled to represent the visible gene, thus fulfilling Belling's dream of the visible gene (which he had already believed to come true). If no corpuscular gene

exists, as is indicated by so many facts, the rhythmical series of the bulbs would somehow contain the secret of the polarized genetical pattern of the chromosome. But neither the chemical nor the morphological data, thus far, permit of a final statement (see also below). We hope that Caspersson or someone having the necessary apparatus will apply the ultraviolet technique to this problem in combination with the urea-alkali technique, which ought to clarify some of the facts. Until such a time it is of no use to speculate further in chemical terms. We refrain therefore from discussing the ideas of Gulick (1939) or others on the chemical structure of the chromosome. The chemist Calvin has presented an interpretation of the thermal increment of lampbrush formation for which Kodani furnished the experimental data (Calvin and Kodani, 1941) and for which we must leave the responsibility to him.[3] But we ought to register our doubt in one chemical hypothesis advanced by Calvin in that paper, which is formulated in disregard of the visible morphological facts. Calvin assumed that the chromosomes are constructed with a micellar arrangement of polypeptide chains which are held side by side by weak primary hydrogen bonds and possibly by other stronger bonds, together with the tetranucleotides (nucleic acid) which are attached to the basic side chain amino acids of the polypeptides by means of the salt link formed by the guanidine group of the latter and the phosphoric acid of the nucleotides. On the basis of this assumption Calvin considered that the formation of the lampbrush chromosomes by alkali is the result of the breaking of, first, the hydrogen bonds and then the other stronger bonds holding the polypeptides together (for detail see Calvin and Kodani). In other words, Calvin considers that all the nucleic acid stays attached to the polypeptides during

[3] The junior author has since repeated these experiments, substituting the urea-alkali mixture for pure alkali. The activation energy of lampbrush formation turned out to be about 5 Kcal. as compared with about 8 Kcal. in the former experiments. This suggests caution in regard to conclusions upon the underlying reaction.

treatment. In our opinion, however, the lampbrush chromosomes are not formed by such a process but first by the detachment of some nucleic acid from the disks and subsequent attachment of this freed nucleic acid to the chromonemata, the polypeptide chains of which remain intact at least in the skeleton structure (for further detail see Kodani). Our opinion seems to be supported by the finding of Stanley and others that when the viruses are treated with urea or alkali the nucleic acid is freed from the proteinic component before the protein is broken down.

Mazia and Jaeger have shown that the treatment of chromosomes with nuclease removes all the nucleotides (see Mazia, 1941) without changing the coherence of the chromosome. We reported above that Kodani's experiments showed that actually chromonema and bulbs or hairs remain intact in this case and that therefore there is morphological evidence for Mazia's conclusion that the nucleotides are not needed for keeping the chromosomal structure together (Koltzoff's work on the oocyte-chromosomes shows the same). We do not feel qualified to discuss the problem of the chemical attachment of the nucleotides to the protein skeleton (see Mazia's just-discussed protamine hypothesis), but we want to point out that the experiments have shown a very different chemical or physical behavior of different chromatic parts of the chromosome all of which give the Feulgen reaction. We may distinguish (1) chromatin which probably fills the spaces between the bulbs or cones in the normal disks, which may, however, be derived from a part of the perultimate chromomeres. This Feulgen positive substance migrates under the influence of alkali towards the chromonemata and attaches itself to them. (2) Chromatin of the bulbs (main part of the disks) which remains within the bulbs with short alkali-urea treatment. It also remains within the lampbrush hair, though its granular appearance here indicates a change under prolonged alkali treatment. (3) The heterochromatin, which is completely dissolved immediately in alkali, both in the lamp-

brush hairs and bulbs and the chromocenter, when all other chromatin remains in its place. For this heterochromatin we have Caspersson's analysis indicating the presence of much histonelike protein and in addition some indication of the presence of a ribose-nucleic acid. Again, we must hope for a new attack with Caspersson's methods after the Kodani-treatment. (4) The chromatin of the telomeres which resists the acid alcohol treatment longest for some unknown reason. It might be added that the arrangement of the skeleton and chromatin in the disks makes it necessary to reconsider all the work done by Wrinch, Schmidt, Caspersson, Astbury and their collaborators on the arrangement of the nucleotide molecules in the chromosome in relation to the protein chain. The meaning of all these facts can be understood only after a chemical explanation, especially of the method of attachment of the nucleic acid to the skeleton protein, is available.

A word may be said regarding the heterochromatic sections within the salivary chromosomes which were identified by Kodani (1941) with the lampbrush method and by Prokofieva (1939) with the intrachromosomal-attraction method. The initial presence of the lampbrush structure in the heterochromatic sections, before it is dissolved, shows that the *visible* skeletal structure in these sections is the same as all along the chromosome. This does not mean that the *chemical* structure of the skeleton in the two regions is identical. According to Caspersson, the protein of the heterochromatic sections is of the histone type; this might be arranged in the same visible skeletal structure as the euchromatic skeleton. The skeleton in the heterochromatic regions is easily dissolved by alkali when the skeleton of euchromatic regions is still intact. It is possible, however, that the skeleton in both cases is a protamine chain with the histone occurring elsewhere. The weak bands of the chromosome may be the coils of this chain plus bulbs with little nucleic acid; the histone making up the undifferentiated protoplasmlike mass visible in some such regions. In the heterochromatic regions

there must be some difference between the central chromonemata and the skeleton of the hair or bulbs, since the latter is dissolved here completely when it is still intact in the euchromatic regions. This difference must not necessarily be a simple chemical difference in the skeleton. The attachment of the nucleic acid might also be concerned. The visible difference between the eu- and heterochromatic regions may therefore be based upon the chemical constitution of the skeleton (chromonema and hair), the amount and importance of interdiskal material, and the amount or type of attachment, or both, of the nucleic acid. But it has been impossible thus far to check upon the morphology of the heterochromatin because in the decisive stage, namely, that with coils and bulbs, there is no chromatin left in the heterochromatic parts and the skeleton of the bulbs is also dissolved or invisible. Thus we can not say whether the faintness of the bands and the special structure of some of the heterochromatic regions (the bulblike inflations of the whole chromosome with only vague structural details) in the untreated chromosome is skeletal, or based purely upon the arrangement of the skeleton or upon features of both skeleton and chromatin. Other unknown structures might also be involved. It is thus far difficult to relate structure and chemical aspects of the heterochromatin as studied by Caspersson and Schultz (see Schultz, 1941). Schultz (1941) in his most recent work met with corresponding difficulties. A word may be added about the chromocenter which might give some information not available for the heterochromatic regions within the chromosomes. In the formation of the lampbrush stage the chromocenter is completely and very quickly dissolved. In the formation of the bulb stage it begins to disintegrate right at the bulb stage (see Kodani, 1942). At this stage the structure of the chromocenter is clear and the absence of the spiralized structure is easily observable; nucleic acid bulbs similar to those of the euchromatic regions are present and form four undulated strings per chromosome which are associated side by side and look like an actual

continuation of the chromosomal structure.[4] When disintegration sets in within the chromocenter these undulated strings are broken and the bulbs are set free. The haematoxylin staining does not give any indication of the presence of connecting skeletal threads between the bulbs.

One of the typical features of the salivary chromosomes is the attraction disk by disk which has made it possible for Painter to introduce these chromosomes as a genetic tool. This fact again involves the difficult problem of point by point attraction between homologous chromosomes. The physicist Delbrück (1941) assures us that no physical force is known which could account for a long-distance attraction and that therefore an attraction at an atomic distance at one point with a subsequent zipper action must be assumed. Without entering this discussion, we want at least to point out a few facts which must be taken into account in future discussions. In the experiments on salivary gland chromosomes two different types of attraction became visible. One is the attraction between two perultimate chromomeres of the same chromosome. Both in the lampbrush and the bulb stage we find the hairs or bulbs always in pairs and attached to each other lengthwise. These pairs are the perultimate chromomeres of two chromatids. But the attraction between two halves of a disk is not attraction of perultimate chromomeres but of two half-coils of chromonema, probably folded molecularly into a half cylinder, in the center of the disk. The coiling is in opposite directions in the two synapsed chromosomes but in the same direction in the two chromatids of the same homologue. This relation in the coiling of the four chromatids (though without relation to the chromomeres and to the real structure of the chromosome) has been noticed previously by Frolova (1937) in the development of the salivary nuclei as mentioned above. It is not quite clear whether Kaufmann (see discussion in Metz, 1941) made

[4] This point was not mentioned in Kodani (1942) but it is visible in the photo, figure 8c.

the same observation. The mirrorwise attachment of the bulbs to the chromonemata of the two homologues suggests some basic structural difference between the homologues, for which models might be available. It is premature to speculate upon this point, but these facts may be useful later in forming definite notions.

We turn now to the cytogenetic consequences. One of the goals of the cytogeneticist is to relate the experimental unit, the mutant locus, generally called a gene, to definite chromosomal structures. The experimental work on chromosome breaks as related to the visible structure of the salivary chromosome seemed thus far to take care of the minimum extension of the visible unit of structure. Darlington (1939; see 1942) speaks of "units of X-ray breakage, which provide the physical definition of a gene." If all breaks are between disks, as experience thus far shows, the individual band is this unit. The locus of many point-mutations has been narrowed down by this method to a single band, and some enthusiastic geneticists even call a band a gene. The lower limit of the "visible unit," then, ceases to be a problem if those conclusions stand. The senior author has repeatedly called attention to facts (see 1940) which do not agree with such a conclusion, and Muller (see 1938) has done the same. During the past years we have checked upon the cytogenetical situation at the left end of the X-chromosome of *D. melanogaster* which is the best-analyzed section of all. We constructed a map showing all the breaks available to us with their phenotypic effects and their relation to the mutant loci. The conclusions to be drawn from such a map were partly discussed in Goldschmidt (1940).[5] Meanwhile Demerec (1941) has independently done the same work and, as he had at his disposition a much larger collection of breaks, his elaborate data make ours unnecessary. The factual results are naturally the same, as the breaks used by us are also contained in Demerec's much larger collection (except one

[5] This map was shown to many visiting geneticists and has been used in lectures on the nature of the gene.

new small inversion with the left break 5 bands right of yellow). Demerec, in addition, extended his work farther to the right than we had done thus far. The facts are absolutely clear: On both sides of a mutant locus a considerable region with a variable number of bands produces the phenotypical effect of the mutant locus, whatever kind of a break occurs. These regions even overlap. As the regions in question are very well analyzed, one might assume that all mutant loci are known. Thus the visible unit is not one band but a segment of chromosomes, of varying length, including sometimes many bands and overlapping with the following segment under certain conditions (see Demerec, 1941). The facts, then, are perfectly clear, though their interpretation is controversial, as expected, with Demerec preferring an interpretation in terms of a single gene with a more or less large area of action (which, by the way, does not explain why this action is that of the *mutant* locus), the senior author preferring an interpretation without any corpuscular gene. This is not the place to discuss this problem. The point which we want to make here is that in a purely physical sense the unit has increased far beyond the minimum as expressed above in Darlington's words. It is worthwhile to call attention to the possibility that the small number of leptotene chromomeres as compared to the large number of perultimate chromomeres may mean that the leptotene chromomeres correspond to the different smaller or larger segments just discussed.

The present work on the structure of the salivary chromosome shows now that at the other end, the upper limit, the situation is comparable: The minimum of one band is no longer a minimum. As a band is at least one coil with about half a dozen perultimate chromomeres, many unknown physical, *i.e.*, visible things might happen within a band. Deficiencies or inversions are imaginable as hitherto unknown features of the perultimate chromomeres. Stadler (1941) has recently again summarized how experimental work has shown all transitions from the visible chromatin rearrangement to the not visible

point mutation. Sax once pointed out that it is not very important to discuss (in this connection) what is beyond visibility. The present insight however makes it all-important to study now the cytogenetics of the uncoiled disks. We are trying to develop a method for such a study, which is hardly possible with any of the methods thus far used. The future will show what can be done. But already now we can point to a few genetic facts which lie in the direction of our search. Some time ago the senior author found a new Notch deficiency which he gave to Gottschevsky, his assistant at that time, for a cytological check. The most careful and repeated study showed that the perfectly clear genetical deficiency for quite a section including facet was not accompanied by any chromosomal alteration (see Gottschevsky, 1939). Gottschevsky assumed therefore that no physical deficiency was present but an "inactivation of genes," whatever that might mean. Demerec (1940) has since found other Notch deficiencies of the same type and has accepted Gottschevsky's interpretation. To the same chapter belongs Oliver's (1937) genetical analysis of facet, which makes him suspect a deficiency though the chromosomes are normal. Here is material which might give surprising results when the proper method is found for a study of uncoiled individual bands.

Finally the work of Schultz and Caspersson with ultraviolet absorption might come in here and lead to a solution of the physical (visible) basis of the so-called point-mutations. They claim that differences of nucleic acid content within a band are associated with genetic changes. As soon as a reliable technique is available the same problem may be attacked cytologically by analysis of intra-disk structure.

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CHANGES IN THE DEGREE OF DOMINANCE OF FACTORS AFFECTING TAIL-LENGTH IN THE HOUSE MOUSE

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MENDEL coined the term dominance to designate the expression in the heterozygote of one of a pair of contrasted characters, to the exclusion or nearly complete exclusion of the other or recessive member. Most students of genetics now agree that the degree of expression of the two members of a pair of alternative or allelic factors is determined, not only by properties specific to the members of this pair, but by the whole setting in which the development of the character occurs.

One of the important features which determines this setting and thus influences the dominance relation is the rest of the genetic constitution of the individual. It is known, for example, that in certain strains of fowls the mutant factor "rumpless" is dominant, while the same factor transferred to a different strain is almost wholly recessive. Similar instances are known in a variety of other animal and plant species. In a few cases, the results have been shown to be due to interaction between two identifiable factors, as in the relation between the mutants semi-forked and forked in Drosophila, as described by Lancefield (1918); or between the frizzle mutation in the fowl and its recessive modifier (Landauer, 1933). These interactions result in dominance modification, but since the expression of the character affected is generally modified in the homozygous mutant forms as well, it seems best to regard such modification of dominance as a special case of the more general phenomenon of factor interaction.

Cases of dominance modification deserve more analytical study than they have received, for they may give the opportunity to discover the developmental reasons for

dependence (or non-dependence) of the effects of one factor upon those of another, and may contribute directly toward the solution of problems concerning the origin of dominance in evolution. Fisher in particular (1928, 1930) has focussed attention on the latter question through his hypothesis of the evolution of dominance through the natural selection of dominance modifiers. This hypothesis assumes a specific relation between the modifier and the mutation whose dominance is affected. An alternative view has been suggested by several investigators (*e.g.*, Muller, 1932; Plunkett, 1933; Dunn and Landauer, 1934, 1936) that the "modifiers" are selected for their own effect on development. The acquisition of dominance by the wild-type allele is thus held to be incidental to the selection of factors which enhance the expression of normal characters generally. This hypothesis does not require specific relationships between mutants and dominance modifiers but leads rather to an expectation that "dominance modifiers" are merely factors with general effects on the phenotypes which are subject to modification. Thus, reverting to the examples first cited, any factor which tends to enhance the development of the tail vertebrae in the fowl might be expected to reduce the dominance of a mutation such as rumplessness.

One way to test such ideas is to compare the effects of the same "modifying" factors on two different mutations leading to the same or a similar phenotype. In breeding experiments with several different mutations affecting tail length in mice, such a comparison was obtained and it led to the surprising result that the same group of factors appeared to modify two phenotypically similar mutations in opposite directions.

Description of Material

The two mutations studied are known as Brachyury and D-short. Both are treated as dominants and are lethal when homozygous. The former was found as a mutant by Dobrovolskaia-Zawadskaia (1927) and has

been extensively studied by her and by Kobozieff (1935). Its most marked effect is a shortening of the tail, often accompanied by other distortions and malformations of the tail. It was originally reported as a dominant and in most laboratory stocks of *Mus musculus* the heterozygotes are readily distinguishable from normals, having at birth tails from one fourth to three fourths of the normal length, often with a fine threadlike filament at the end. At origin, however, the mutation probably had relatively little phenotypic effect. Zawadskaia *et al.* (1934) have reported that the Brachyury stocks are descended from two males which had been treated with x-rays. One of these animals bred as a heterozygote for a Brachyury mutation, hence the mutation had occurred in an ancestor and was not connected with the radiation. In fact this male was later found to have a slight kink at the end of a tail which was only slightly shorter than normal. He produced animals with slight effects like himself and others with shorter tails. The other treated ancestor had a tail of normal length, but among his grandchildren some short-tailed animals were found which proved to be heterozygous for the same Brachyury mutation. The stock from which both of these males were descended was that of the Institute Pasteur in which previously Dubosceq (1922) had found animals with hereditary tail abnormalities. It is unlikely that the same mutation occurred independently in these two males; it is more reasonable to suppose that there was no connection between the radiation treatment and either of the mutations. (Zawadskaia has assumed no connection in the case of the first male.) It is probable that the mutation to Brachyury had occurred some time in advance of the treatment but having little phenotypic effect (in some animals, none at all) it escaped detection until crosses of the progeny of radiated animals produced new modifier combinations, and a careful hunt for anomalies disclosed the mutant forms. Once found, those animals would be selected for breeding which showed the character in a

pronounced form and since only heterozygotes are viable the selection would be restricted to heterozygotes and a degree of dominance thus brought about. The probable steps in the acquisition of dominance in this case thus agree very well with those predicted by Fisher (1928) on the theory that dominance results from the effects of selection for stronger expression of a character in heterozygotes.

The same steps have probably been followed by breeders of fancy mice in bringing about the enhancement of a number of mutations which produce the bizarre or unusual forms desired by fanciers. Thus the dominance of variegated spotting (Dunn, 1937) was clearly brought about by selection; and yellow coat color probably shows "fanciers' dominance" also, since when backcrossed repeatedly to an unselected stock approximating the genetic constitution of the wild house mouse, the heterozygous yellows are hardly distinguishable from non-yellows. The apparent lability of dominance of Brachyury is thus not exceptional or peculiar among mouse mutations.

The D-short mutation was reported as a dominant with recessive lethal effect by Dunn, Gluecksohn-Schoenheimer and Bryson (1940). The original short-tailed animals had been found by Dr. C. H. Danforth in his laboratory stocks, and noting the resemblance to the Brachyury mutation, he sent them to us for comparison and testing with the older mutation. Although phenotypically similar, this mutation proved to be quite independent of Brachyury. It has behaved as a strong *dominant* in the several combinations and stocks of *Mus musculus* in which we have bred it, the heterozygotes being either tailless or having short tails, seldom more than half the normal length.

Dominance Modification of the Brachyury Mutant

The first attempts to influence the expression of Brachyury were made by Dr. Paul Chesley and myself in 1933–4 with the primary purpose of increasing the via-

bility of the homozygotes which Chesley (1932) had found to die regularly at the end of the tenth day of development. We carried out two series of breeding experiments. The first was intended to test the effect of heterosis on the viability of homozygotes and to study the expression of the Brachyury mutation when introduced into the widely different genetic backgrounds provided by the different inbred lines of our laboratory and by the species *Mus bactrianus*;[1] the second to determine whether factors which modify the heterozygote toward normal also modify the homozygote. Although our chief object was to extend the life of the homozygote and thus to make possible a study of further and later morphological effects of the homozygous condition, we also carried out systematic selection experiments with heterozygotes and attempted a genetical analysis of the factors concerned in tail-length modification and of their effects upon the homozygote. These experiments had been virtually completed at the time of Dr. Chesley's death in 1936 and the data had been partially analyzed.

Observations on Homozygotes

To study the effects of heterosis on homozygotes we compared the time of death and the external characters of 51 homozygous embryos from the original Brachy stock obtained from Dr. Dobrovolskaia-Zawadskaia in 1930 and since inbred in our laboratory, with 74 homozygotes obtained from outcrosses between the Zawadskaia stock and other unrelated inbred lines of normal mice. Of the latter, 39 embryos were obtained from crosses involving at least three different inbred lines, and at least three generations removed from the original Zawadskaia stock. Thus in this category is litter number 77 dissected at 10 days 8 ± 6 hours containing 12 normal embryos and 2 typical homozygotes which had just died. The mother was a Brachy, from a cross of two different lines: (a) her father from the third backcross of Brachy to our inbred

[1] We are grateful to the Jackson Memorial Laboratory and to Dr. C. C. Little and Dr. C. V. Green for sending us a stock of *Mus bactrianus* in 1933.

line SK, her mother from the second backcross of Brachy to line 1a. The father of litter 77 was a Brachy descended from two backcrosses of Brachy to *Mus bactrianus*. The maximum heterosis should have been obtained in crosses involving four lines, that is a $\times$ b; c $\times$ d; followed by ab $\times$ cd. Although only six homozygous embryos from such crosses were examined they showed no marked deviation from other homozygotes. There was a remarkable uniformity throughout in the time of death of homozygotes. This occurred at the end of the tenth day after copulation. The homozygotes with hybrid constitution failed to survive appreciably longer than those with inbred constitution nor were gross morphological differences apparent.

Two of the inbred lines were found to have factors which greatly decreased the effect of the Brachy mutation on tail length. Certain animals containing such factors had tails of normal length, although progeny tests showed them to be heterozygous for *T*. These occurred frequently in backcrosses of Brachys to the *bactrianus* stock; and thus provided good material for the question concerning the effect of such modifiers on homozygotes. Such tested normal-tailed heterozygotes from the "*bactrianus* Brachy" stock were bred together and the pregnant females dissected at the end of the tenth day. The 11 homozygotes found did not differ in appearance or in time of death from those obtained from shorter-tailed heterozygotes. In this as in the previous comparison, the methods were such that small effects of the modifiers on homozygotes would escape detection, and since timing of embryos was accurate to $\pm$ 12 hours small differences in time of death could not be established. It can safely be said, however, that regardless of parentage all homozygous embryos examined were in a similar stage of development and showed a similar complex of traits (*cf.* Chesley, 1935). Even when the heterozygous effect of *T* had been suppressed, therefore, the homozygous effect appeared not to be altered. Since for our purposes, suffi-

cient answers had been obtained to our questions concerning homozygotes we did not pursue them further with selected lines differing in tail length.

EFFECTS OF MODIFIERS ON HETEROZYGOTES

The outcrosses of Brachys of Zawadskaia's stock to several inbred lines showed that the length of tail in the Brachy heterozygotes obtained from such crosses depends on the heredity of the line to which the cross is made; while the production of lines differing in tail length by selection from the Brachy stock, and the results of crosses between such selected lines similarly showed that a part of the variability in tail length accompanying this mutation was due to other factors, and not, as Zawadskaia had at first supposed, to instability of the Brachy gene. At the time of our experiments these facts had not been established and we therefore attempted to identify and study some of the factors modifying the expression of Brachyury.

In the meantime, however, Green (1936) had shown, by outcrossing a Brachy stock to an unrelated strain of the same species and to *Mus bactrianus,* that in the former case at least one, and in the latter, several factors modifying (increasing) the tail length in the heterozygotes ($T+$) were introduced from the unrelated stock; that differences in tail length of the resulting lines were clearly genetic and that some of the variability of non-inbred Brachy stocks is probably due to factors modifying the expressivity (dominance) of *T*. Kobozieff and Kobozieff (1939) have also shown that there are genetic differences between stocks of Brachys differing in tail length. Thus parents with shorter tails produce a higher proportion of entirely tailless (extreme Brachy, type I) young than parents with tails of intermediate length (type II); while Brachy parents with longer tails (type III) produce none of these extreme variates. Whether the three Brachy types recognized by the Kobozieffs as I, II and III constitute different inbred lines with limited

ranges of variation is not apparent from the description given. The longer-tailed Brachy parents, on the other hand, produced a significant excess of *normal* (not-Brachy) offspring. The authors seem not to have noted this last-named fact.[2] It probably means that some Brachy heterozygotes were indistinguishable from normal; and thus that the longest tailed parents contain factors which minimize or suppress the expression of *T* in heterozygotes. The same interpretation is probably to be placed on the difference observed by Kobozieff (1935, p. 352) between the results of crossing Brachys (tailless and short) with normals from their own stock (242 Brachy : 242 normal) and with normals from unrelated normal stocks (133 Brachy : 189 normal; P = .04). The unrelated normals probably introduced factors tending to suppress the expression of *T* as observed also by Green and by us.

Our outcrossing experiments confirmed the conclusions that factors exist which modify the degree of expression of the Brachyury mutation, but since they did not lead to identification of such factors nor to analysis of their mode of action they need not be discussed in detail. They were, however, obtained in a somewhat different way and with different materials from those used by others and are hence briefly recorded below.

Length of tail at birth was taken as the most convenient measure. The tail of each mouse was measured at birth with a millimeter rule, using the level of the anus as the starting point. In Table 1 are shown the effects on tail length of outcrossing the original Brachyury stock, descendants of animals kindly supplied by Dr. Zawadskaia in 1930, with three representative unrelated normal stocks and of backcrossing Brachys from such crosses to the unrelated stock. The effect in each case is to *increase* the tail length of the Brachy offspring. The effect of the *bactrianus* stock is greater than is shown by the criterion in

[2] The offspring of all Brachy × Brachy matings involving a Brachy of the longest tail length class (III) were 275 Brachy, 203 normal, expected 319 : 159; deviation = 44 ± 10.3; p = > .001.

TABLE 1

COMPARISON OF TAIL LENGTH AT BIRTH OF Z BRACHYURY STOCK (OBTAINED FROM DR. ZAWADSKAIA) AND OF ANIMALS DESCENDED FROM OUTCROSSES OF THIS STOCK TO NORMAL STOCKS

	Number of animals	Mean tail length in mm		Length ratio Brachy to normal	Absolute difference from Z Brachy	Diff. S.E. Diff.
		Brachys	Normals			
Z Brachy	101	6.0 ± .26	13.3	.45		
Bagg Brachy*	27	7.6 ± .57	13.3	.57	1.6 ± .63	2.5
la Brachy†	19	10.6 ± .32	13.3	.80	4.6 ± .41	11.2
F_1 Z Brachy × *M. bactrianus*	17¶	8.8 ± .53	11.0	.80	2.8 ± .59	4.8
BC F_1 ZBr/*bact.* × *M. bactrianus*	11	9.0 ± .77	11.0	.82	3.1 ± .81	3.8
BC F_1 ZBr/*bact.* × Z Brachy	25	7.3 ± .69	13.3	.55	1.3 ± .74	1.8

* From three backcrosses of Z Brachy to inbred Bagg albino stock.
† From three backcrosses of Z Brachy to inbred line la (pied).
¶ Eleven of these not phenotypically distinguishable from normal at birth.

the last column, since normal *bactrianus* has a tail length some 15 per cent. less than that characteristic of our normal *musculus* stocks. The difference between normal and Brachy in *bactrianus* is thus less than between normal and Brachy even in a *musculus* stock with plus modifiers of Brachy. The last line shows that when *bactrianus* hybrids are backcrossed to the Zawadskaia Brachy stock, tail length tends to return to the lower mean characteristic of the original Brachy stock.

The segregation ratios of Brachy to normal were also studied in outcrosses to six different inbred lines, namely, Bagg albino, Little's dilute brown, SK. 1a (inbred pied lines of this laboratory), 19a (inbred "all-white" line) and *M. bactrianus*. F_1 Brachys from these outcrosses were backcrossed to the normal inbred parent line, and in the case of several lines the process was repeated through two subsequent backcrosses. The Brachy animals of the first, second or third backcross were then bred together and crossed with Brachys from other lines for the heterosis experiment already reported. A total of some 2,100 progeny were observed. No significant departures from the expected ratios of Brachy to normal were noted except in crosses to line 1a and to *Mus bactrianus* which, as already shown, contain factors which reduce or suppress the expression of the Brachy mutation. In crosses to each of these stocks and in matings of animals derived from them, a deficiency of Brachys and an excess of normals was found. Thus from backcrosses of Brachy to line 1a 153 young were observed of which only 40 (instead of 76.5 expected) showed any effect of the Brachy mutation at birth and these in general had tails of nearly normal length.

A striking effect of outcrossing was noted in the interspecific cross to *Mus bactrianus*. An F_1 of 32 individuals was observed. At birth only six instead of the 16 expected could be classified as Brachy. Of these two had a tail length $\frac{7}{8}$ that of normal, two were $\frac{3}{4}$, one $\frac{5}{8}$ and only one was short, with a tail $\frac{1}{8}$ of normal length. As the F_1

animals grew older, more of them could be identified as $T+$ by the development of a small hook (due to fusions of most distal vertebrae) at the end of the tail. At weaning eight animals had developed this hook. By progeny testing all the normal-appearing animals three more were found to be heterozygous for Brachyury. Thus, the actual ratio of animals with the mutation to those without it was 17 : 15, a nearly perfect 1 : 1 ratio. Similar results were obtained in subsequent backcrosses. Thus out of six lines tested, two (one *musculus* line and a *bactrianus* stock) contained factors which suppressed the effect of *T* in some of the offspring. Factors in other lines did not have sufficiently marked effects to cause deviations from expected phenotypic ratios.

Effects of Selection on Heterozygotes

From the descendants of crosses of the original Brachyury stock with the various inbred lines two selection lines were set up. In one of these, designated Line S, the Brachys with shortest tails, in the other (Line L) those with longer tails were selected for breeding. The pair from which the L line was descended contained blood of Zawadskaia Brachy, and of SK, 1a, dbr, 19a, Bagg and *bactrianus* stocks, and the tail length of this pair was $\frac{3}{8}$ and $\frac{1}{2}$ of normal, respectively. Matings in this line were exclusively brother by sister for ten generations. The "S" line originated in related animals ($\frac{3}{8}$ and $\frac{1}{8}$ tail length) with blood of Zawadskaia, SK, 1a and Bagg lines but none from *bactrianus*. When a few completely tailless animals appeared in the early generations, an attempt was made to breed these together, so that the matings were at first not exclusively brother by sister. Many of the tailless animals proved to be sterile and later exclusively brother-sister matings were made between siblings with shortest tails (usually not tailless). The two lines, which at first were similar in respect of tail length of the Brachy animals became quite different as shown by comparison of contemporary generations in

Table 2. At the end of ten generations of selection in "L" line and of six generations in "S" line there was but little overlapping in the tail-length distributions of "L" and "S" lines. Selection was undoubtedly effective and made rapid progress in the short line; in the "L" or long selected line it was much less effective and this line changed but little between the early and the later generations. Tail length was nearer its upper limit in "L" line at the start of the experiment, and thus less progress was possible; while in the "S" line, it is probable that recessive factors were being selected and progress was therefore more rapid.

TABLE 2

DISTRIBUTION OF TAIL LENGTH IN SELECTED LINES OF BRACHY MICE AND HYBRIDS BETWEEN THEM. TAIL LENGTH IN MM AT BIRTH.

	0	1	2	3	4	5	6	7	8	9	10	11	12	n	m
Line "S" F_{3-6}	58	16	16	6	3	5	2	1						107	1.14 ± 1.6
Line "L" F_{8-10}			1	1		3	5	5	5	8	12	12	2	54	8.4 ± 3.3
F_1 "S" × "L" .	7	4	9	6	12	16	15	11	9	12	9	1		111	5.6 ± 2.75
F_2	28	12	23	15	19	22	37	16	15	12	12	5	1	217	4.8 ± 3.1

Brachys from each of these lines were then crossed with the same inbred normal stock (Bagg albino) and the tail length of the Brachys in the two F_1 generations compared. The 33 F_1 Brachys from "L" line (F_{10}) had a mean tail length at birth of 9.2 ± 1.2 mm; while 49 F_1 Brachys from "S" line (F_6) averaged 6.5 ± 2.8 mm. When the distributions of the two F_1's were compared by χ^2, they were found to differ significantly from each other ($\chi^2 = 30.4$, $n = 12$, $P = .001$).

Brachys from the two selected lines S and L were then crossed together reciprocally and the tail length at birth of the Brachys in F_1 and F_2 was measured (Table 2).

The F_1 Brachys were extremely variable, but in distribution and in mean tail length they were about intermediate between the parental forms. In the F_2, the mean tail length was about the same as in F_1, but segregation occurred as indicated by the appearance of many tailless and very short-tailed Brachys which are never found in the "L" line. However, there is no comparable mode representing the longer tails of the "L" line.

The fit of the actual F_2 distribution to a distribution calculated on the assumption of a single modifier factor difference, without dominance, between "S" and "L" lines gives $P = 0.132$. Such an hypothesis therefore is not excluded by the data but we made no accessory test of its validity such as the result of inbreeding the extreme variates from the F_2. Green (1936), from an F_2 distribution showing less evidence of bi- or trimodality than ours, concluded that one main incompletely dominant modifier differentiated a normal inbred stock (dbr) from the Brachy stock used by him. The existence of independent factors modifying the expression of Brachyury is incontestable in Green's excellent data on vertebral numbers, and in his data as in ours these factors are probably few in number; but in view of the transgressive non-genetic variation to which the Brachyury tail length and form is subject, it can not be concluded from either set of data that a single modifier has been identified.

Our observations concerning modifications of the heterozygote agree with those of Green and of Kobozieff. In the outcrosses of Brachy to normal strains reported by all three investigators the effect has always been to *increase* the tail length of the heterozygotes. This effect has been shown to be due to the genetic constitution of the particular strain to which the cross is made. Apparently, different inbred stocks contain modifiers with different quantitative effects upon the tail length of Brachy heterozygotes, but in all known cases these factors tend to modify the heterozygote toward the wild type, that is, they tend to make the Brachy mutation recessive. The existence of such modifying factors is shown also by the effectiveness of selection in producing strains differing in the tail length of Brachy heterozygotes. Such strains may differ by one or a few factors modifying tail length.

MODIFICATION OF THE D-SHORT HETEROZYGOTE

After these experiments had been completed, the new short-tail mutation, *Sd,* came under observation. In the

stock in which it had occurred, the heterozygote had a short tail of from $\frac{1}{8}$ to $\frac{1}{3}$ of the normal length. The homozygotes in all combinations which we have observed are completely tailless, have many abnormalities and always die shortly after birth.

Heterozygotes were outcrossed to the same normal-tailed Bagg albino inbred stock to which the Zawadskaia Brachy stock had been crossed. Heterozygous F_1 animals were again backcrossed to Bagg albino and the process repeated through five successive backcrosses. The object was to obtain a stock as nearly isogenic as possible with the long inbred Bagg stock, and containing the *Sd* mutation. A Brachy stock isogenic with Bagg albino had already been obtained and it was desired to study the interaction of Brachy and D-short with other genes held constant. In the preparation of this Brachy stock it had been found that the length of the Brachy tail tended to increase somewhat with increasing concentration of genes of the Bagg stock. Table 1 shows the effects of the first three backcrosses of Brachy to Bagg stock. Eventually tail length in this stock, although still somewhat variable, came to average about $\frac{3}{4}$ of the normal length.

As backcrossing of D-short to the Bagg stock proceeded, it was noticed that the tail length of the heterozygotes was decreasing and the proportion of heterozygotes which were entirely tailless was increasing. In the fourth backcross generation, for example, out of 94 heterozygotes observed 57 either had no tail at all or a very short stump. The changes in the proportion of tailless animals during six generations are shown in Table 3. By BC_5 80 per cent. of all heterozygotes were tailless. The maximum length of tail also decreased until in BC_5 no animals had tails longer than about $\frac{1}{3}$ of normal. There can thus be no doubt about the striking decrease in tail length which accompanied the increasing concentration of the genes of the Bagg albino stock.

This effect occurred against the pressure of selection. It was found that tailless heterozygotes suffer a much

TABLE 3

DISTRIBUTION OF TAIL-LENGTH TYPES AMONG D-SHORT HETEROZYGOTES IN THE ORIGINAL STOCK AND IN SUCCESSIVE BACKCROSSES TO THE BAGG ALBINO STOCK

	From matings of Sd + by ++		
	++	Sd +	
	Normal	Short	Tailless
Danforth stock (P_1)	39	39	8
$F_1 \times$ Bagg nt	59	52	3
BC_1	42	22	7
BC_2	53	36	19
BC_3	173	91	79
BC_4	118	37	57
BC_5	109	22	80
Total	593	552	

higher mortality (Dunn, Gluecksohn-Schoenheimer and Bryson, 1940) than normals or short-tailed animals, while those which survive are frequently sterile. Heterozygotes with short tails were therefore preferred and more likely to be available for mating, but the decrease in tail length occurred in spite of this. Moreover, the direction of change was reversed when, in order to save the stock from extinction, heterozygotes were outcrossed to a different normal-tailed inbred stock. The F_1 reared from the outcross of Bagg D-short by normal consisted of 42 normals, 35 with short-tails ranging up to $\frac{1}{3}$ of normal length and only 5 tailless. In the stock derived from inbreeding and backcrossing these D-short animals most of the heterozygotes have tails varying from a short stump to nearly $\frac{3}{4}$ of the normal length. The reduction in length in the backcrosses to the Bagg stock was thus due to genes which are characteristic of the Bagg strain. These genes increased the expression (dominance) of the *Sd* mutation. It was this same stock (Bagg albino) which had previously been found to contain genes which decreased the dominance of the similar Brachyury mutation.

DISCUSSION

The decrease in dominance of the Brachy mutation when crossed to several normal stocks may be attributed to the presence in the normal stocks of factors tending to increase tail length and to decrease the effect of the fac-

tor *T*. It is simplest to conceive of these as unmutated wild-type genes, which are not, however, the same in all strains. Whether their presence is to be explained by Fisher's hypothesis as due to the repeated recurrence of the Brachy mutation in the species, or whether such genes have been selected for their own effect apart from the occurrence of such a mutation can not be settled from the present evidence. It is to be noted that genes with the strongest effect in decreasing the dominance of the Brachy mutation were found in a species (*Mus bactrianus*) other than that in which the mutation occurred. The *bactrianus* genes, as Reed (1937) has shown, have an even stronger inhibiting effect on the expression of another *musculus* tail mutation, Fused, a mutant like Brachy. The presence and distribution of such genes in wild species and their physiological effects on development are questions of considerable interest to which the present data contribute little.

The question which may be most profitably discussed in the light of these new experiments concerns the nature of the interaction between tail mutations and dominance modifiers whereby the genes of the same stock are able to influence the Brachy and the D-short mutations in different ways.

One may assume that such interactions will depend not merely on the type of character, that is, the completed phenotype, but primarily on the way in which the particular phenotype is attained during development. Although Brachy and D-short appear to represent the same phenotype, the short-tail is reached in two different ways. In the case of the Brachy heterozygote, Chesley (1935) showed that the failure responsible for the resorption of the distal part of the tail during embryogeny is localized in the notochord. In the ten-day Brachy embryo the notochord does not extend throughout the tail. Its end is marked by a sharp constriction, distal to which the somites undergo progressive resorption until by the 15th or 16th day only a filament remains to represent the distal

end, while the shortened tail ends bluntly. In the D-short heterozygote, the tail also appears to be normal until about the 11th day embryo. Then a vascular breakdown indicated by the formation of a bleb or haematoma begins at the end of the tail, and the distal part degenerates and disappears before birth (Dunn, Gluecksohn-Schoenheimer and Bryson, 1940, fig. 3). In both cases the mutant phenotype is reached through pathological processes which destroy parts which have already been differentiated, but the processes affected by the two short-tail mutations are not the same.

If in phenotype we include the processes by which it is reached, then these two phenotypes are different and respond differently to the same group of factors. Genes from the Bagg stock apparently increase the severity and extent of the abnormal process leading to vascular breakdown and thus accentuate the effect of *Sd*, but they tend also to promote the development of the tail notochord and thus to diminish the effects of *T*. These two effects may thus be assumed to occur through different channels.

When two mutations operate through different channels, their effects are liable to modification by different factors, and each will thus appear to have dominance modifiers specific to itself.

In the present case we may conclude that these dominance modifiers show some specificity for particular mutations rather than for general phenotypes. The specificity probably inheres in the developmental processes by which the phenotype is reached.

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DIFFERENTIATION OF OLD AND NEW WORLD SPECIES OF THE GENUS *COLUMBA*[1]

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THIS investigation was made in an effort to determine whether there are morphological criteria that distinguish the species of *Columba* that inhabit the Old and New World, respectively.

MATERIALS AND METHODS

In order to have easily available the ranges of the many pigeon species, their geographic distributions were sketched on outline maps of the World (Nystrom Series of Desk Maps, No. DD9, World). These distributions were taken largely from Peters' work (1937).

Through the kindness of Mr. Rudyerd Boulton and Mr. H. B. Conover, the authors were permitted to examine the extensive collections of the *Columba* species in the Field Museum of Natural History, Chicago. The chief object was to determine whether the many species could be arranged into large groups in terms of their morphological characteristics and geographical distributions. Naturally, there were many specific qualities which had no value for these purposes. But as one looks over the collections, certain striking morphological features appear, in greater or lesser intensity, in a number of species. These were the characteristics to be studied, for it was readily apparent that some of these obvious criteria were restricted to those pigeon species which inhabited a single continent, whereas others distinguished the birds of other continents. In compiling the data regarding the various morphological characters considered in this paper, more than two thirds of the species listed by Peters were used. With some of the characters studied, data for practically all the species were available.

[1] Paper No. 282 from the Department of Genetics, University of Wisconsin, Madison, Wisconsin.

Aside from observing characters on the animals themselves, a few taxonomic criteria were taken from the literature. In the main, Salvadori's (1893) catalogue was used, although in some instances it was necessary to check the systematics in more recent works. When it was possible to do so, the data taken from the literature were checked with specimens.

Results

Color of Underparts: Of the more obvious criteria which distinguished the Old from the New World species of *Columba,* the color of the underparts is one of the most striking. The many species were arranged in a graded series in terms of the relative amounts of gray, purple or brown present in the feathers of their underparts. The categories that were used were: "white or light gray," "dark gray or black," "light purple-gray," "dark purple-gray" and "brownish purple or brown" underparts. When this arbitrary arrangement was made, the species of the Old World were found to be predominately gray, while in the species of the New World purple and brown predominate. Table 1 presents the data concerning color of underparts. In this table the species are grouped into two large categories, in terms of the relative amounts of gray (Column 3) and purple and brown (Column 5). One must understand, in considering Table 1, that this categorical presentation is but relative, and was designed only for the purposes herein stated. In placing the species in their respective categories, effort was made to observe all the specimens under much the same light; in this way, changes due to different illumination were minimized.

In Table 1, one may see that a single species sometimes occurs in two categories. Thus *C. palumbus* is found among the species with "light gray" and "light purplish-gray" underparts, and accordingly occurs in both columns. Duplications appeared because there actually were representatives of the species in each of the two categories.

TABLE 1

COLOR OF UNDERPARTS OF *COLUMBA* SPECIES

Range	Total no. species	White, light gray, dark gray or black underparts		Light purple-gray, dark purple-gray, brownish purple or brown underparts	
		Species	Number	Species	Number
Old World	19	guinea livia oenas oliviae palumbus rupestris unicincta leuconota albitorques norfolciensis pulchricollis trocaz vitiensis janthina jouyi	15	palumbus delegorguei iriditorques vitiensis punicea	5
New World	17	leucocephala maculosa squamosa	3	albilinea caribaea fasciata gymnophtalmos inornata oenops picazuro rufina araucana flavirostris plumbea goodsoni nigrirostris purpureotincta	14
Columns	2	3	4	5	6

Practically all the species listed as having white, light gray, dark gray or black underparts are residents of the Old World. *C. maculosa, leucocephala* and *squamosa* are the only New World forms found in these categories. On the other hand, the forms listed as having light or dark purplish-gray underparts are predominately from the New World. *C. palumbus, vitiensis, delegorguei* and *iriditorques* are the only Old World exceptions. The species with "brownish-purple" underparts, likewise, include a preponderance of New World forms.

Thus, one may conclude that purple is a color most often characteristic of the underparts of New World pigeons, whereas shades of gray are more frequently observed among the Old World species. With reference to brown, there is conflicting evidence. The species *C. vitiensis, iriditorques* and *punicea* are the only Old World forms that have brownish-purple underparts. However, many

individuals of the species *punicea* have underparts which are almost entirely brown, with no apparent trace of gray. In the large majority of cases, nevertheless, the brownish species are found in the New World. It is apparent, therefore, that the *Columba* species may be more or less roughly divided into Old and New World forms, in terms of the color of their underparts.

Color pattern of the tail feathers: The color pattern of the tail feathers of all the New World and Old World pigeon species described by Peters was considered. The species in each geographic unit may be divided into at least three categories, to wit:

(1) Twelve of the New World species possessed tail feathers that were of uniform color. That is, they were either uniform black, gray or brown. There was no evidence of banding. This group included the following species: *leucocephala, gymnophtalmos, flavirostris, oenops, inornata, speciosa, nigrirostris, goodsoni, subvinacea, plumbea, chiriquensis**[2] and *purpureotincta.* (2) A large group of New World species possessed tail feathers that had a relatively light terminal band across the tail. In some of the species this distinct terminal band was brownish gray, in others bluish brown, and it varied in width from 3 to 6 cm. All the species of this group had black or very dark coloration of the tail feathers, proximal to the light terminal band. This group of species included *squamosa, caribaea, albilinea, araucana, fasciata* and *rufina.* (3) The last group of New World species included only *picazuro* and *maculosa;* these two forms had terminal bands on their tail feathers, but the band was darker, instead of lighter, than the proximal region of the feather. In both species the band was black, whereas the proximal area of the feather was gray.

(1) Eighteen of the Old World species had tail feathers of uniform coloration; practically all of them were slate black. This group included the following

[2] Those species marked with an asterisk (*) were not observed in the museum. Data regarding them were taken from the literature, mainly from Salvadori (1893).

species: *hodgsonii, arquatrix, thomensis**, *albinucha**, *elphinstonii**, *torringtoni**, *pulchricollis**, *punicea**, *palumboides**, *janthina**, *versicolor**, *jouyi**, *vitiensis, pallidiceps**, *norfolciensis, pollenii**, *delegorguei,* and *malherbii**.[3]

(2) Another group of eleven Old World species possessed a distinct subterminal band on the tail feathers. This band was lighter than the regions distal or proximal to it, and in some instances the subterminal band was pure white. The dark terminal region was similar to the terminal band in the New World forms, and was usually found to be from 2 to 5 cm in width. The light, subterminal band was clearly defined and varied from 1 to 5 cm in width. This group of species included: *leuconota, rupestris, oenas, oliviae, albitorques, palumbus, trocaz, junoniae**, *unicincta, guinea* and *argentina**.[4]

(3) This Old World group included only the two species, *livia* and *eversmanni.* These forms were characterized by a black terminal band, with a lighter proximal area of the tail feathers. The markings of these two species were similar to those of the New World species, *maculosa* and *picazuro.* Some *livia* individuals have a thin, light tip to their tail feathers. This is not to be confused with the wide terminal bands herein mentioned.

(4) A final Old World group included only the single species: *iriditorques**. This species is the only one of the Old World forms which has a terminal band distinctly lighter than the proximal region of the tail feather. In this species the terminal band is of yellow buff, and the proximal region is a slate gray.

Summarizing the data relative to the color pattern of the tail feathers of the *Columba* species, we may make the following generalizations: (1) Most of the species from both hemispheres have tail feathers of uniform color.

[3] Of this group, data were not available for *jouyi, thomensis* and *albinucha;* these were placed in this category on the basis of their close relation to other members of the group.

[4] *Columba junoniae* was provisionally placed in this category by virtue of its relation with *C. trocaz.* Data were not available.

(2) The other New World species with two exceptions have a terminal band that is lighter than the proximal area of the tail feather. (3) The two exceptions in the New World forms have terminal bands that are darker than the proximal regions of the feather. (4) Most of those Old World species that do not have uniform tail feather coloration have a subterminal band that is lighter than the regions proximal and distal to it. (5) There are three exceptions to this last generalization: two species have terminal bands that are darker than the proximal region, and one species has a terminal band that is lighter than the proximal region of the feather. (6) Perhaps the most obvious conclusion to draw from a consideration of the color of the tail feathers of the *Columba* species is that there is probably a fundamental band pattern that exists in all the species. In some species the band is darker proximally, and in others it is darker distally. In the great majority of species, however, the uniformly dark color is so intense that it has blanketed any appearance of the band pattern.

Table 2 shows, in summary form, the number of species that possess each of the various plumage characters considered herein.

Mantle, scapulars and wing-coverts earthy-brown: As in the case of the underparts, the mantles, scapulars and wing-coverts of the New World species are characterized by the presence of brown. Of the species examined, 19 were New World forms and 27 were Old World forms. Ten of the New World *Columbas* have earthy-brown mantles, scapulars and wing-coverts, while only one Old World form is so recorded. This one Old World species is *C. pollenii,* a specimen of which was not available at the Field Museum. Consequently, it was impossible to check the coloration along with the other species, and the inclusion of *pollenii* among the brown species was made by literature reference only. The New World species that were marked with earthy-brown mantles, scapulars and wing-coverts included: *goodsoni, gymnophtalmos,*

maculosa, nigrirostris, oenops, picazuro, plumbea, purpureotincta, speciosa and *subvinacea.*

"Scaly" feathers on the hind neck: Six of the New World species have peculiar color patterns on the feathers of the hind neck. Each feather is bordered with a velvety black, maroon or metallic green band, in such a way that the neck feathers, as a whole, present a characteristic scaly appearance. This condition has not been observed in any of the Old World *Columba* species. The New World species that have this characteristic are: *leucocephala, picazuro, gymnophtalmos, squamosa, speciosa* and *albilinea.*

TABLE 2

NUMBER OF SPECIES POSSESSING EACH PLUMAGE CHARACTER

Plumage character	Number of Old World species	Number of New World species	Total number of species studied	
			Old World	New World
White, light gray, dark gray or black underparts	15	3	19	17
Light purple-gray, dark purple-gray, brownish purple or brown underparts	5	14		
White or gray subterminal band across tail, 1″–2″ from tip of the feathers	10	0	32	20
Light gray terminal band across the tail feathers	1	6		
Dark gray or black terminal band across the tail feathers	3	2		
Uniform dark tail feathers	18	12		
Neck feathers of "scaly" appearance.	0	6	17	19
Mantles, scapulars, and wing-coverts earthy-brown	1(?)	10	27	19
Wing-coverts with dark bands or patches	6	0	27	19

Wing-coverts with dark bands or patches: Of the 19 New World species examined, none had dark bands or patches on the wing coverts. However, more than one fifth of the Old World species were so distinguished. These Old World forms were: *albitorques, eversmanni, leuconota, livia, oenas* and *rupestris.*

Other plumage characteristics: There are other plumage characters which, if given more thorough study, might be found to distinguish the Old from the New World species of *Columba.* For example, cursory examination revealed that from one fourth to one half of the Old World forms

were grayish-white on the throat and beneath the bill; while only about one tenth of the New World species had this marking. Similarly, both Old and New World species have metallic patches on the nape. However, among the New World *Columbas* the patch is well defined and quite distinct in about half the species; while there are much fewer Old World forms that are so marked, and the marking differs in size from that observed on the New World species. Closer study of these and other characters will probably reveal a distinct difference in the frequency with which these characters occur in the Old and New World species, respectively.

Discussion and Conclusion

Apparently, therefore, the *Columba* species may be segregated into Old and New World groups on the basis of plumage characters. Although this analysis does not permit of an entirely clear-cut differentiation, nevertheless it is evident that certain genes that affect plumage color are distributed in the Old and New World species, respectively, in significantly different frequencies. The plumage relationships are shown in Fig. 1.

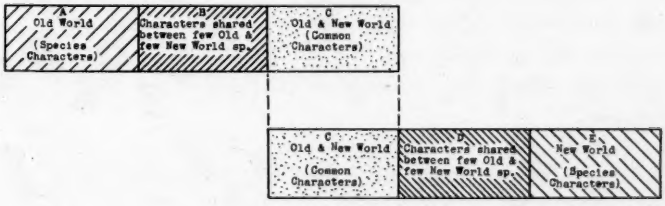

Fig. 1

Category A of Fig. 1 represents the plumage characters limited exclusively to the species of the Old World. These characters include (1) white or gray subterminal band across tail, 1″–2″ from the tip of the feathers, and (2) wing coverts with dark bands or patches. These qualities are found only in the Old World species, as pointed out earlier. There are, naturally, many species

specific characters which would fall into this plumage class, that have not been treated in this discussion.

Category B of Fig. 1 includes plumage effects which are contained in a few but not all Old World species, but which are shared with a few New World species. The characters from which the B category is derived are (1) white or light gray underparts and (2) dark gray or black underparts. Eight Old World and 3 New World species possess white or light gray underparts, and 9 Old World and 2 New World species possess dark gray or black underparts.

Category C of Fig. 1 represents all those plumage characters which are held in common between the species of the two continental masses, such as, for example, the uniform coloration of the tail feathers, represented in 18 Old World and 12 New World species. This category, likewise, will include many characters which serve to distinguish the genus *Columba* from other genera.

Category D may be represented by the following characters: (1) "light purple gray underparts," (2) "dark purple gray underparts." All these characters are represented in nearly one half of the New World species but in less than one sixth of the Old World *Columbas.* Further, the characteristic "light gray terminal band across the tail feathers" might be placed into this category, as it is found in six New World and one Old World species.

Category E was determined by virtue of: (1) "earthy brown mantles, scapulars and wing-coverts," and (2) "neck feathers of 'scaly' appearance."

Thus, it is evident that we can segregate the Old from the New World *Columbas,* on the basis of morphological differences.

Let us now consider this material in relation to other data that have been gathered in this laboratory. It has been possible to separate eleven of the *Columba* species into two broad categories, the Old and New World species, respectively, in terms of the antigens present in their

erythrocytes. (Irwin and Cumley, 1940; Cumley and Irwin, in preparation). Although by this immunological procedure the separation of the species into these two great groups is not entirely clear-cut, nonetheless it appears that the geographic division is reasonably sound. Briefly, the antigens that affect this segregation may be defined as follows:

(1) "Common" antigens, shared in different combinations among many New and Old World *Columba* species.
(2) "Group" antigens, shared among many, if not all, of the Old World species, but not found in the New World species.
(3) "Group" antigens, shared among many, if not all, of the New World species, but not found in the Old World forms.
(4) "Species" antigens, which include those characteristic of each species of Old World pigeons.
(5) "Species" antigens, which include those characteristic of each *Columba* species of the New World.
(6) Antigens contained in a very few, not in all Old World species, but also found in certain New World species. This is a relatively insignificant category.
(7) Antigens found in a very few, not in all, New World species, but also found in a certain few Old World species. This, likewise, is a relatively insignificant category.

These last two categories have been included here on the basis of meager evidence. Were it not for them, we could assert that by virtue of the immunological procedures, and for the particular species investigated, any New World *Columba* species is more similar to any other pigeon species of the New World than it is to any Old World form. Likewise, any species of the Old World is more like any other pigeon of the Old World than is any species of the New World. But if these two categories, (6) and (7), become well established by further tests, these conclusions will be untenable. In any event, however, the reactions that determine these two categories are thought to be exceptions to the rule, and do not detract from the more or less sharp differentiation of the Old and New World species, respectively.

Apparently, therefore, the immunological and morphological methods of study have yielded similar results. Both demonstrate: (1) "common" characteristics shared

by both Old and New World species; (2) characteristics shared by a few Old and a few New World species; (3) characteristics found only in New World species; and (4) characteristics found only in Old World species.

It would appear that the morphological differentiation on the basis of plumage coloration, as well as the immunological differentiation, are dependent upon the relative frequency of certain genes or gene-complexes in the species inhabiting the different continental masses. This does not imply, of course, that the genes causing distinctive plumage differences also determine the immunological differentiation of the Old and New World species.

One other matter of interest should be mentioned. A study has been made of the relation of size to geographic distribution of the *Columba* species (Cumley, in press). This investigation revealed that a greater proportion of the species having shorter average total length, wing length, tail length and tarus length inhabit the warmer regions of the earth. In the cooler regions there is a preponderance of the large forms. Thus, it appears that Bergmann's Rule, (see Allen, 1905), or some modification of it, may be applied to the species of the genus *Columba*.

In so far as size is concerned, then, evolution appears to have proceeded primarily latitudinally, whereas in the matter of plumage colors and antigenic differences, the species seem to have evolved longitudinally.

SUMMARY

Species of Old and New World pigeons were differentiated on the basis of (1) color of underparts, (2) color pattern of the tail, (3) mantles, scapulars and wing-coverts earthy-brown, (4) wing-coverts with dark bands or patches and (5) "scaly" neck feathers.

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BIOMETRIC COMPARISON OF SEVERAL SAMPLES, WITH PARTICULAR REFERENCE TO RACIAL INVESTIGATIONS

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IN comparing series of frequency determinations biologists have found inadequate or cumbersome the methods that are in general use and are described in the numerous text-books on statistics. In a table one may present, for each sample of the biometric series, the frequency distribution and the ordinary statistical information: number of specimens, total range of variation, mean, standard deviation and standard error (the probable error, 0.6745 times the standard error, adds little to the interpretations and at the present time is commonly not computed). Without further computations, however, such a table does not indicate the differences between the means for each two samples, or the significance of such differences; nor does it readily and clearly test the consistencies of the values for related samples, or the trend of the means. The difference between the mean of a sample and that of any other sample may of course be computed and tabulated, and the averages may be graphed. Presenting all such material, however, may often carry the work beyond the limits of practicability in the way of time or of publication.

Dice and Leraas' method[1] for presenting comparative data on a series of samples consumes less time in preparation and less space in presentation than the usual methods, and has distinct advantages in that the graphically presented results may readily be interpreted and compared. The method here proposed, and exemplified in Fig. 1, is virtually the same, except that we have also plotted the standard deviation (σ), as a measure of dispersion. It is often of vital importance to consider dis-

[1] *Contr. Lab. Vert. Gen., Univ. Mich.*, 3: 1–3, 1936.

persion, too, since by using enough variates one may reduce so greatly the standard errors of the means (σ_M) for two samples that differences of practically certain statistical significance will be demonstrated between the means of samples, even though the overlap of frequency distributions is so great that it would be absurd to dis-

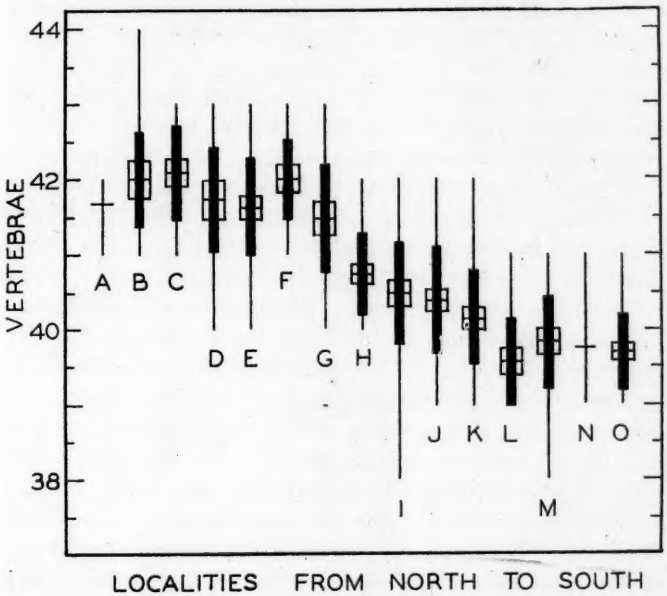

FIG. 1. Variation from north to south in the number of vertebrae of the anchovy, *Anchoviella mitchilli*.

A, Massachusetts (3 specimens); B, Great Peconic Bay, New York (25); C, Hudson River, New York (50); D, Great South Bay, New York (30); E, New Jersey north of Cape May (63); F, Cape May, New Jersey (30); G, Mount Landing, Virginia (48); H, Charleston Harbor, South Carolina (77); I, Georgia (64); J, eastern Florida (95); K, western peninsular Florida (77); L, St. Andrews Bay, Florida (50); M. Texas (47); N, Río Papaloapam, Mexico (8); O, Yucatán (100). See text for a description of the graph.

tinguish the populations so represented, at least in systematic studies. Except for errors due to insufficient size of samples, values for σ do not vary with the number of specimens used, whereas values for σ_M vary inversely with the square-root of the number of variates.

For each sample the graph (Fig. 1) shows: (1) the total range of variation, by a vertical line; (2) by the broad portion of the line, one standard deviation on each side of the mean; (3) by the hollow rectangle, twice the standard error on each side of the mean; and (4) the mean, which is indicated by the cross-bar.

When two samples showing normal variation are compared, an overlap in the frequencies of about 16 per cent. only is indicated when the broad bars representing the standard deviations neither overlap nor are separated on the ordinate scale. That is, about 84 per cent. of the specimens of both categories would then be separable, in that their values for the given character would lie on the proper side of the line best separating the two populations. Such differences are approximately of the order that may be utilized for subspecific separation.[2]

The significance of the difference between any two means becomes graphically apparent by comparing (1) the relative length and (2) the relative overlap or separation of the corresponding rectangles (delineating, for each sample, 2 σ_M on either side of the mean). As Dice and Leraas indicated, "significance" (in the usual, arbitrary interpretation of biologists), is approximately indicated when the rectangles just meet, end to end. This is a special case; we need some indication too of the significance of the differences (t values), corresponding to the varying relative lengths, and to the varying overlap or separation, of the rectangles. This desideratum we have supplied in Table I. The t values are the critical ratios: difference between any two means, divided by the standard error of the difference. The standard error of the difference, as here used, is represented by the conventional formula

$$\sigma_\Delta = \sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}$$

Most of the critical ratios were first determined empirically, but they have been checked and extended by special formulas applicable to this case:

[2] Ginsburg, *Zoologica*, 23: 253–286, 1938.

$$t = \frac{2(1+y-2x)}{\sqrt{1+y^2}}, \text{ when the rectangles overlap;}$$

$$t = \frac{2(1+y+2x)}{\sqrt{1+y^2}}, \text{ when the rectangles are separated.}$$

In these formulas, derived for us by Charles W. Cotterman,

x = relative overlap or separation of the rectangles in terms of the length of the shorter one.
y = ratio of length of longer rectangle to that of the shorter one.

It will be seen from Table I, supplemented by Table II, that considerable reliance can be placed on the significance of the difference between samples, if the corresponding rectangles are only slightly separated, or if the overlap is not more than about 33 per cent. of the length of the shorter of the two rectangles. When the longer rectangle is 2 to 4 or more times as long as the shorter one, an overlap of 50 per cent. or even 75 per cent. does not negate the probability that a significant difference exists. If the gap between the rectangles exceeds 10 per cent. of the length of the shorter, the significance of the difference between the populations may be regarded as demonstrated beyond any reasonable doubt (if we assume the samples to be representative).

When the overlap of two subequal rectangles exceeds one third of the length of the shorter rectangle, little or no reliance can rightly be placed on the significance of the difference. When the overlap is 50 per cent. the odds of significance are less than 10 to 1 unless the length of one rectangle is more than 4 times that of the other. Such differences in the values for σ_M are very rare in comparable data. Ordinarily any very considerable overlap renders the significance of the difference very doubtful. If the shorter rectangle is included wholly within the height of the other, an overlap of more than 100 per cent. is indicated and no significance should be attributed to the difference between the means of the two samples.

The method here presented is applicable to any series of samples in which multiple comparisons are desired in terms of range, deviation, standard error and mean. It is particularly valuable, however, in comparing samples

TABLE I

CRITICAL RATIOS (t VALUES) OF THE DIFFERENCE BETWEEN THE MEANS OF ANY TWO SAMPLES TO THE STANDARD ERROR OF THE DIFFERENCE, FOR USE IN THE INTERPRETATION OF MATERIAL COMPARED GRAPHICALLY BY THE METHOD HERE DESCRIBED

		Relative lengths of rectangles (y)				
		1:1	1.5:1	2:1	4:1	∞:1
The longer rectangle	100 per cent.	0.0	0.6	0.9	1.5	2.0
overlaps the shorter	75 " "	0.7	1.1	1.3	1.7	2.0
rectangle by the	67 " "	0.9	1.3	1.5	1.8	2.0
given percentage	50 " "	1.4	1.7	1.8	1.9	2.0
(100 x) of the length	33 " "	1.9	2.0	2.1	2.1	2.0
of the shorter	25 " "	2.1	2.2	2.2	2.2	2.0
rectangle:	10 " "	2.5	2.5	2.5	2.3	2.0
The two rectangles neither overlap nor are separated	(0 per cent.)	2.8	2.8	2.7	2.4	2.0
The longer rectangle						
is separated from the	10 per cent.	3.1	3.0	2.9	2.5	2.0
shorter rectangle by	25 " "	3.5	3.3	3.1	2.7	2.0
the given percentage	50 " "	4.2	3.9	3.6	2.9	2.0
(100 x) of the length	100 " "	5.7	5.0	4.5	3.4	2.0
of the shorter rectangle:	200 " "	8.5	7.2	6.3	4.4	2.0

which are naturally seriated. Thus the data in Fig. 1 test for one species the tendency of the vertebrae to decrease in number toward warmer waters (in this case from north to south); and also the statistical significance of the trend. It is at once clear from the presentation that the northern

TABLE II

PROBABILITIES THAT DIFFERENCES ARE DUE TO CHANCE, AND THE CORRESPONDING ODDS OF SIGNIFICANCE, FOR REPRESENTATIVE RATIOS (t VALUES) OF THE DIFFERENCE BETWEEN TWO MEANS TO THE STANDARD ERROR OF THE DIFFERENCE

These computations assume that the curve of variation is normal. Table II supplements Table I.

Ratio of difference between means to standard error of difference (t)	Probability that difference is not significant	Odds of significance
1.0	.317	2 to 1
1.2	.230	3 to 1
1.3	.194	4 to 1
1.4	.161	5 to 1
1.5	.134	6 to 1
1.6	.110	8 to 1
1.7	.089	10 to 1
1.8	.072	13 to 1
1.9	.057	16 to 1
2.0	.045	21 to 1
2.1	.036	27 to 1
2.2	.028	35 to 1
2.3	.021	46 to 1
2.4	.016	60 to 1
2.5	.012	80 to 1
2.6	.009	106 to 1
2.7	.007	143 to 1
2.8	.005	195 to 1
2.9	.004	267 to 1
3.0	.003	369 to 1
4.0	.000	15,770 to 1
6.0	.000	500,000,000 to 1

populations have a higher average number of vertebrae than do the southern ones, and that the end series are connected by intergrading populations. Differences of probable significance distinguish adjacent collections both in the north and in the south, but the local deviations are not great enough to negate the essential integrity of the northern and southern types as contrasted with one another. Two subspecies plus intergrades are thus indicated. The erection of an intermediate subspecies on the basis of the number of vertebrae is not justified, in view of the gradual change in the central region and the wide overlap between the values for the intergrades and those for the forms on either side (as indicated by the position of the black bars which represent the standard deviation).

Many series of samples will not show the regular pattern of variation exhibited in Fig. 1, but the method is also adapted to illustrate such heterogeneous variation.

Fig. 1 shows not only the trend of the variation, but also strongly confirms the validity of the distinction of the northern and southern forms, by reason of the consistency of the results. Even when no single sample of one group can be demonstrated as significantly different from any sample of another group, a true distinction between the two categories must nevertheless be accepted, if the difference is shown consistently by enough small samples of each group.

Adequate sampling of populations, for such comparisons as are here discussed, often rests more on biological judgment than on statistical considerations. Life-history and ecological information are often particularly significant. As pointed out by Ginsburg (*loc. cit.*), variational figures are of more significance, at least in systematics, if they are drawn from heterogeneous material. The true average values for a form may well be missed, if the entire sample comes from one time and place. Single samples may be far from representative, on account of spatial and temporal variations. It is obviously most desirable to have numerous samples from different local-

ities, habitats and seasons, not only to obtain an adequate idea of local variation but also to smooth the data for each form and to arrive at approximately true means for its characters. Most illuminating results are apt to accrue, when a study is made of material which represents the form throughout its geographical and ecological range. If the results obtained from series of samples are consistent enough, and if the variations in the ecological conditions bring a few populations of one form under the environmental conditions normal to the other form, it will often be possible to determine whether observed differences are of environmental rather than genetic origin.

SUPPLEMENTARY STATEMENT ON FORMULA TO BE USED IN DETERMINING THE STANDARD ERROR OF THE DIFFERENCE BETWEEN TWO MEANS (σ_Δ)

After preparing this paper we found that Simpson and Roe[3] (pp. 192–194) had recently indicated a method for computing the standard deviation of the differences between two means (σ_Δ), which, they claim (pp. 316–320), largely invalidates the Dice–Leraas method of comparing samples or calls for an involved estimate of the reliability of that method when the number of specimens (N) in the two samples is dissimilar. They recommend that the generally accepted formula

$$(1)\quad \sigma_\Delta = \sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}$$

should generally be replaced by formula

$$(2)\quad \sigma_\Delta = \sqrt{\frac{N_1}{N_2}(\sigma_{M_1})^2 + \frac{N_2}{N_1}(\sigma_{M_2})^2}$$

Formula (1) is properly used, they point out, when the populations are discrete; that is, in determining whether there is a significant difference between the means for any character in two species (or in any populations which do not intermingle or interbreed). Formula (2) is indicated as proper when it is desired to test the assumption that two samples could have come from a homogeneous population. They hold that this is the assumption that would

[3] "Quantitative Zoology." New York and London: McGraw-Hill Book Co., 1939.

ordinarily be put under test by the Dice–Leraas graphical method.

Whether to apply the conventional formula (1) or the new formula (2) in determining the standard error of the difference between two means (σ_Δ) would often be a difficult decision, to be arrived at only on an arbitrary and subjective basis. Ordinarily, however, the determination of σ_Δ will be made for two populations which are probably but not certainly discrete. The degree of interbreeding or intermingling between the populations may vary from 0 to 100 per cent., and will seldom be subject to even remotely accurate determination.

The modified formula proposed by Simpson and Roe is not to be recommended for use when the evidence indicates, with certainty or high probability, that there is little or no interbreeding between the two populations being compared. This evidence may consist in knowledge of the individual movements between the habitats of the two populations, or in character differences that would certainly or probably not exist if there were any very extensive intermingling. These differences may lie solely in the character under comparison, but will commonly rest on or be confirmed by differences in other characters, or on ecological considerations. In general, when using the graphical method of comparing the statistics for a series of populations, there will be involved units that are probably distinct, to a large degree. In so far as the evidence indicates the discreteness of the populations, the use of formula (1) is actually to be preferred, in such comparisons as those here under discussion.

In general there will be little advantage, on statistical grounds, in using formula (2) rather than (1). Simple inspection will show that the values of σ_Δ will be identical if $N_1 = N_2$, and it can also be shown that the values for σ_Δ will be identical if the standard deviations (σ) of each sample are the same. The σ_Δ values will not be significantly different if there is only a moderate difference in the values either of N or of σ. Ordinarily one of these

two items will be of the same order of magnitude in the two populations, and that is all that is required to give results that are not essentially different for biological comparisons.

That the use of formula (2) would not usually give values for σ_Δ critically different from those obtained by the use of formula (1) is further indicated by a random empirical test (Table III) of the data behind Fig. 1. In this table the values for σ_Δ are computed for each comparison of samples (except for sample A, based on 3 specimens, and for sample N, based on 8), first by the use of formula (1) and then (in parenthesis) by the use of formula (2). Most of the differences in the values obtained by the two methods are wholly immaterial and none are large enough to alter any biological conclusions to be drawn from the computations.

If we would need apply formula (2) rather than formula (1), the graphical comparison of the samples here employed would become too cumbersome to be of utility, in so far as the indications of significance between differences of the means are concerned. Simpson and Roe propose a table which, on the basis of the use of formula (2), would make it possible to estimate roughly the reliability of the graphical indications of the significance of these differences, in accordance with variations in the ratios of N_1 to N_2 as well as of σ_{M_1} to σ_{M_2}. But their table as drawn up applies only to the special case in which the two rectangles in the graph neither overlap nor are separated. To cover different degrees of overlap and of separation, as we have done in Table I, other tables would be required, and this would complicate the graphical method beyond the point of practicability.

Simpson and Roe's proposal to add "the theoretic ranges ($M \pm 3\sigma$)" to the Dice–Leraas graph is considered unwise. The extra line clutters the picture and adds little to its interpretive value. On account of biological factors, extreme limits in the range of variation are hardly predictable in the variable characters of animals,

TABLE III

COMPARATIVE VALUES OF σ_Δ AS DETERMINED BY THE USE OF FORMULAS (1) AND (2), FOR DATA SHOWN IN FIG. 1

The determinations made by the use of the formula (2) proposed by Simpson and Roe are given in parentheses. These may be seen to differ little from those obtained by the formula (1) usually employed. The letters refer to the populations sampled.

	B	C	D	E	F	G	H	I	J	K	L	M
C	.158 (.157)	...	...	...	...	...	...	...	...	...	...	...
D	.177 (.177)	.150 (.149)	...	...	...	...	...	...	...	...	...	...
E	.153 (.152)	.120 (.120)	.144 (.142)	...	...	...	...	...	...	...	...	...
F	.158 (.154)	.127 (.136)	.150 (.150)	.120 (.132)	...	...	...	...	...	...	...	...
G	.164 (.167)	.135 (.134)	.156 (.158)	.128 (.126)	.135 (.145)	...	...	...	...	...	...	...
H	.143 (.129)	.108 (.104)	.134 (.122)	.100 (.098)	.108 (.111)	.117 (.110)	...	...	...	...	...	...
I	.153 (.152)	.120 (.120)	.144 (.143)	.113 (.113)	.120 (.132)	.128 (.126)	.100 (.098)	...	...	...	...	...
J	.148 (.152)	.114 (.117)	.139 (.141)	.106 (.108)	.114 (.135)	.122 (.121)	.092 (.094)	.106 (.108)	...	...	...	...
K	.148 (.144)	.114 (.113)	.139 (.135)	.106 (.106)	.114 (.126)	.122 (.119)	.092 (.092)	.106 (.106)	.099 (.100)	...	...	...
L	.153 (.146)	.120 (.120)	.144 (.139)	.113 (.115)	.120 (.125)	.128 (.128)	.100 (.098)	.113 (.115)	.106 (.113)	.106 (.108)	...	...
M	.158 (.156)	.127 (.127)	.150 (.148)	.120 (.121)	.127 (.134)	.135 (.135)	.108 (.104)	.120 (.121)	.114 (.118)	.114 (.114)	.120 (.120)	...
O	.139 (.119)	.103 (.095)	.130 (.112)	.094 (.089)	.103 (.103)	.112 (.100)	.078 (.077)	.094 (.089)	.086 (.085)	.086 (.084)	.094 (.091)	.103 (.095)

and are likely to transgress the theoretical limit of 3 σ—as do 2 of the 7 ranges shown in the sample given by Simpson and Roe (Fig. 31, p. 318).

We conclude, on the grounds of desirability as well as of convenience, that formula (1) rather than (2) should ordinarily be used in computing the values for σ_Δ, for either tabular or graphical comparisons of the characters of samples. Formula (2), however, should be used where there is clear reason to suspect that the samples come from a freely intermingling population, but in that event the graphical method here recommended would have too little value to warrant its use.

In the preparation of this note we have profited by the criticism of several statisticians and biologists, including Harry C. Carver, C. I. Bliss, W. D. Baten, Frank W. Weymouth, A. Franklin Shull, Lee R. Dice, Ralph Hile, and, particularly, Charles W. Cotterman. Laura C. Hubbs and Max Woodbury generously assisted in the computations.

SOME CONSIDERATIONS ON THE PHENOMENON OF CHIASMA TERMINALIZATION

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OF the various factors thought to play significant roles in the phenomenon of chromosome movement and behaviour, none, perhaps, has been for so long the object both of experimentation and speculation as that of electrical forces governing attraction and repulsion. This is largely because the cytoplasmic and nuclear configurations attending cell division so suggestively points to the presence of electrical fields of force. There are, however, numerous and serious objections to the acceptance of any of the present explanations to the remarkably accurate and synchronous behaviour of the chromosomes in division (Schrader, 1940). That there are, on the other hand, forces of some kind involved in chromosome movement can not be overlooked. As far back as 1905, Lillie called attention to the fact that the behaviour and disposition of chromosomes in the nucleus during prophase and on the spindle at metaphase imply repellent forces that tend to keep the chromosomes at some distance from one another. Numerous workers since then have accepted this so-called electrostatic hypothesis. Churney and Klein (1937) have demonstrated, in the salivary gland nuclei of *Sciara,* that the chromosomes are electro-negative while the nucleus as a whole is electro-positive. The nuclear charge probably depends on the surface properties of the nuclear membrane, and recently Schrader (1941) has emphasized the importance of this structure in controlling chromosome and centrosome behaviour, his hypothesis agreeing with the data of Churney and Klein in that the chromosomes and the nuclear membrane are opposite in charge. It would seem logical, then, to expect, on a purely physical basis, a certain spatial dispo-

sition of chromosomes in the nucleus with respect to each other and to the nuclear membrane. Darlington has attempted to fit meiotic prophase behaviour into a unified scheme based on specific attractions and non-specific repulsions, these concepts being incorporated in his precocity theory of meiosis and his electrostatic theory of terminalization. It is with the latter theory that this paper will concern itself.

Terminalization, as defined by Darlington, is that movement of chiasmata along the arms of paired chromosomes from their points of origin to more distal positions, complete terminalization being achieved when all chiasmata come to lie at terminal loci. That such a movement does occur has been adequately demonstrated in a variety of organisms, both plant and animal. Likewise, it has been equally well established that the number of chiasmata can decrease as prophase progresses toward metaphase. To account for the observed meiotic configurations, Darlington postulated the existence of two kinds of repellent forces: (1) a special charge, localized at the centromere, and (2) a generalized, less effective, charge concentrated on the surface of the chromosome throughout its entire length. The resultant movement of chiasmata is in a distal direction because of the greater repulsion existing between the centromeres, allowing the chiasmata adjacent to the centromere to gain at the expense of the more distal ones until an equilibrium is reached between adjacent loops, or until all the chiasmata have been completely terminalized. The degree of terminalization is thus conceived to be a visible expression of the effectiveness of these forces acting throughout the duration of prophase following chiasma formation. In addition, it is stated that the generalized repulsion is "one having a constant effect in all organisms, the other a varying effect in different organisms" (Darlington and Dark, 1932.) Organisms having a relatively strong centromeric repulsion would, therefore, possess a greater degree of terminalization; equilibrium between the centromere loop

and adjacent loops would prevent further movement of chiasmata.

The theoretical existence of electrostatic forces affecting chiasma movement rests upon a number of observations and considerations. (1) "The existence of the distributed surface charge is characteristic of all ampholytes such as nucleoproteins" (Darlington and Dark, 1932). (2) The circular form attained by loops between adjacent chiasmata when equilibrium is reached, and the fact that successive loops come to lie at right angles to each other, implies the presence of some force acting on all parts within the loop. (3) Terminalization is more effective in bivalents with closed loops than in those which have a single chiasma in one arm only. (4) Terminalization is likewise more effective in interlocked bivalents. (5) "The existence of the local charge may be inferred from the commonest static observations such as, for example, the departure from circular form in the equilibrium position of the attachment loop and the exceptional state of tension seen in the chromosome between the spindle attachment and the first (proximal) chiasma whenever these are very close together (as in *Prunus* with small chromosomes and in *Fritillaria Meleagris* with localized chiasmata)" (Darlington and Dark, 1932). (6) The greater expansion, usually, of the attachment loop also implies the presence of a localized force acting within this loop.

There seems to be little doubt among cytologists concerning the presence of a repellent force affecting chromosome distribution, but it should be pointed out that the conception is largely one of convenience and not one of experimental verification. It is known, as stated above, that the chromosomes react electro-negatively, and hence should repel each other, but the difficulty of computing or directly determining the magnitude of the charge makes it impossible to ascertain the distance through which it is effective, and emphasizes the need for caution in making use of it to interpret observed configurations. Again one is led to speculate on the possible role of the

matrix, forming as it does the external sheath of the chromosome, and hence, the probable active repelling surface. One may ask, too, how the phenomena of synapsis in salivary gland chromosomes and somatic pairing in Drosophila, Culex (Berger, 1938), and Spinacia (Gentcheff and Gustafsson, 1939) can be reconciled with the conception of an active repellent force. Furthermore, one of the basic tenets of the electrostatic theory, as in the precocity theory of meiosis, is the initial assumption that single unpaired threads are homologously attracted and that pairs of paired threads repulse, but the untenableness of this assumption is indicated by the numerous reports that pairing and separation of chromosomes and chromatids is not a function of the subdivision of the chromonemata (Hughes-Schrader, 1940). Is the repelling force effective at one stage and not at another? What conditions are a necessary prerequisite for the effective functioning of this force? These questions and others must be answered before one can state with certainty the nature of the mechanisms governing chromosome movement.

One may also discuss, in like manner, the function of the centromere in chromosome movement. Darlington's evidence has been given. Metz (1938), as the result of a study of monocentric divisions in Sciara, looks upon the centromere as a center of some force governing chromosome movement. Cooper (1941) states that the repulsion of the centromeres in meiosis of *Melophagus* takes place prior to metaphase as evidenced from the drawn out connections between paired homologues. The behaviour of acentric fragments points to the centromere as the center of forces governing congression of the chromosomes onto the metaphase plate, while the bipolarity of a bivalent as a necessary condition to regular congression in meiosis is revealed by the irregularity in behaviour or univalents which frequently never reach the metaphase plate. According to Darlington, the centromere must possess some inherent property which gives it its repellent force.

From a recent study in Tradescantia, observations have been made which lead one to question the efficacy of the centromere as a repelling body. It could be clearly shown that the attenuation of the chromonemata between the centromere and the first proximal chiasma, which Darlington and Cooper regard as evidence of centromeric repulsion, occurs *only when the centromeres are on the metaphase plate.* This may be seen in Fig. 1. The chro-

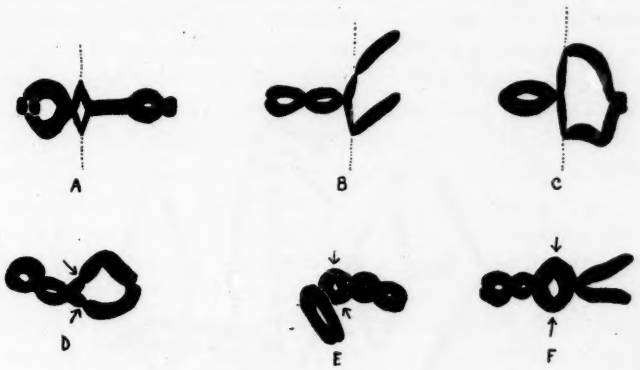

FIG. 1. Chromosomes A, B and C lie on the metaphase plate and show clearly the centromeric repulsion; chromosomes D, E and F are from pre-metaphase figures and show no repulsion. See text.

mosomes have been given a thermal shock, a treatment which prevents the marked chromosome contraction characteristic of this genus under normal conditions, and allows one to study more clearly chromosome structure and behaviour, but which does not impair nuclear division. Chromosomes A, B and C lie on the metaphase plate, and the attenuation of the thread clearly reflects the so-called "repulsion." Chromosomes D, E and F, however, are from pre-metaphase configurations, and although the chiasmata are equally close to the centromere, there is not evidence of a stretching of the threads. Even more convincing evidence of this behaviour is shown in Fig. 2. A bivalent with chiasmata close to the centro-

mere has failed to congress properly onto the plate. According to the electrostatic theory, there should be a departure from the circular loop with a marked pulling out of the threads on either side of the centromere as in figure 1A, but such, it can be readily seen, is not the case. Failure to congress onto the plate has resulted in a failure to show a noticeable repulsion between centromeres. Is

FIG. 2. Lagging bivalent demonstrates lack of repulsion when lying off of the metaphase plate.

one to conclude, then, that repulsion is a property possessed by the centromeres *only when on the plate*[1] (it may well be emphasized that in some organisms terminalization is complete before metaphase) or is the attenuation of the threads and the marked separation of the centromeres a result of the poleward "pull" of the spindle? Furthermore, the observation has been frequently made that lag-

[1] See Hughes-Schrader (1939, pp. 319) concerning behavior of the x-chromosome of Llaveiella.

ging bivalents (as in Fig. 2) often congress later on, to divide and pass to the poles in a regular fashion. Yet these delayed bivalents, comparable in structural development to those which have divided before them, show no evidence of repulsion until they move onto the plate. It seems natural and reasonable, therefore, in the light of these facts, to look upon repulsion as a result of the influence of the spindle on the centromeres and not as a result of an inherent repelling force residing in the centromeres. The next obvious step is to cast doubt on the effectiveness of the centromeres as organs concerned in chiasma movement, as well as on the electrostatic theory of terminalization as a whole, since its validity hinges on the assumption that the centromeric repulsion varies in different organisms, and the greater the magnitude of its repelling force, the greater the degree of terminalization.

The extreme flexibility of the electrostatic theory has led to its general acceptance, for by varying the magnitude of the special force at the centromere to suit the organism in question, the theory could be made to fit any situation, while at the same time the very nature of the postulated forces practically closed the theory to experimental proof or disproof. An avenue of approach, however, can be made through the coiling mechanism as it is known to exist in meiotic cells.

The observation has been made many times throughout cytological literature that the spiralization of the chromosomes in meiosis and the terminalization of chiasmata are parallel phenomena, yet the writer is unaware of any statement considering them as anything but simultaneous events, quite independent of one another. This can probably be accounted for by the fact that a seemingly insurmountable obstacle is immediately encountered when one considers that in some organisms spiralization proceeds as usual, but terminalization is lacking or nearly so. As will be pointed out below, this apparent discrepancy may be accounted for on the basis of the theory of terminalization to be proposed.

Several instances of the correlated behaviour of terminalization and chromosome contraction come immediately to mind. Lesley and Frost's (1927) now classical case of the long-chromosome mutant in *Matthiola incana* showed that as the chromosomes failed to attain their normal state of meiotic contraction the chiasmata re-

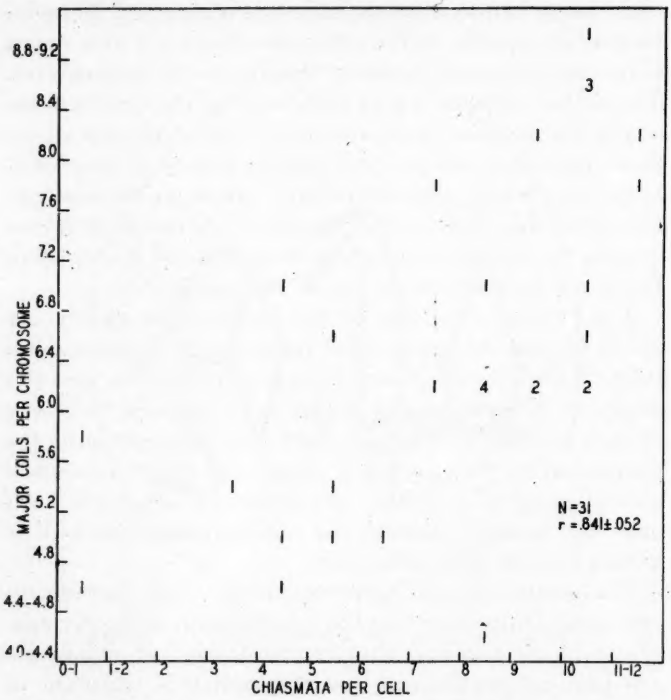

FIG. 3. Correlation table showing the relation between the number of major coils per chromosome and the number of chiasmata per cell.

mained at interstitial loci in contrast to the normal form where the chiasmata are almost wholly terminal and the chromosomes considerably more contracted. Naville and de Beaumont (1933) pointed out that in *Macronemurus appendiculatus*—a Neuropteran insect—the male has chromosomes which are more contracted and chiasmata

which are more terminalized than the female. Similarly, Upcott's (1937) observations in *Lathyrus odoratus* reveal that the normal form has long chromosomes and interstitial chiasmata while the male-sterile mutant presents a picture quite the opposite.

Experimental data (Swanson, 1941) also follow a similar trend. In a recent study of the effects of thermal treatment on major coiling in Tradescantia, a high correlation ($r = .841 \pm .052$) was found to exist between the number of chiasmata per cell and the number of major coils per chromosome (Fig. 3). The data were obtained from plants grown sufficiently long at different temperatures to insure using only those which had undergone the entire meiotic prophase at the desired temperature. A wide range of temperatures (3.5° C. to 42° C.) was utilized for this purpose.

In Tradescantia, the development of the major coils, once they have been initiated, is largely a process of despiralization: the major gyres increase in size and decrease in number as prophase progresses (Swanson, 1941). Although Fig. 3 shows chiasma terminalization and major coiling to be obviously correlated, the correct interpretation of these data is not so evident. Does increased despiralization aid terminalization, or does increased chiasma formation hinder, and slow down, the rate of despiralization? If the latter were correct, one would expect to find that, in *Tradescantia,* where the bivalents are alike morphologically, those bivalents possessing the most chiasmata would show the greater number of major coils, but this is not the case, so it seems most probable that terminalization is directly affected by the despiralization process.

Two other sets of data support the "terminalization through despiralization" hypothesis. It was noticed in some meiotic cells, taken from plants grown at 40° C., that the chromosomes were often found to be in a reduced state of despiralization at metaphase (Fig. 5) as compared to normal (Fig. 4), or as compared to those chro-

mosomes usually found at high temperatures (Fig. 6). The number of "major" coils in these mitotic-like bivalents was frequently as high as thirty-two per chromosome, whereas normally the number of coils is approximately eight for the plant used in this study. Also the chiasmata were slightly higher in number than under normal greenhouse temperature conditions, and a large majority of them were located at interstitial loci as compared with mostly terminal chiasmata in normal cells (Table 1). Also on the same slides bearing these elongated chromosomes (long or l-chromosomes, Swanson, 1941) were found cells containing greatly contracted

FIGS. 4, 5, 6. Fig. 4, normal metaphase. Fig. 5, long-chromosome cell with many coils induced by heat treatments of 40° C. Fig. 6, short-chromosome cell with few coils, few chiasmata and many univalents.

chromosomes (short or s-chromosomes) (Fig. 6). This figure is characteristic of cells grown at this temperature, the chiasmata being largely terminal and the chromosomes short. The same conditions may be induced, it was found later, by subjecting cut inflorescences to 40° C. for 24 hours (Table 1). The coiling mechanism has been greatly altered in some manner by the thermal treatment, and a factor has been provided whose variability further study revealed to be closely correlated with the variability found in the degree of terminalization. In other words, *the number and position of chiasmata at metaphase varies with the degree of meiotic uncoiling.* The slight increase in the number of chiasmata in the long-chromosome cells (grown at 40° C. for 2 weeks) as com-

TABLE 1

CHIASMA FREQUENCIES AT 40° C.

		Interstitial chiasmata per cell	Terminal chiasmata per cell	Univalents per cell	Total chiasmata
Control* (22° C.)		4.14	7.37	0.33	11.51
40° C. (1 day)					
Slide No. 1	l-chrom.	8.45	3.21	0.55	11.66
	s-chrom.	1.96	7.92	1.20	9.88
Slide No. 2	l-chrom.	9.70	2.90	0.72	12.60
	s-chrom.	1.35	8.67	0.80	10.02
40° C. (2 weeks)					
Slide No. 1	l-chrom.	9.50	4.20	0.00	13.70
	s-chrom.	2.08	6.72	1.28	8.70
Slide No. 2	s-chrom.	1.60	7.18	1.33	8.78

* Control = data taken under normal greenhouse conditions; 40° C. (1 day) and 40° C. (2 weeks) = data from plants grown at 40° C. for 1 day and 2 weeks respectively; l-chrom. = long chromosomes and s-chrom. = short chromosomes (see text for explanation).

pared to the control, or normal, cells is probably due to an increased crossover frequency brought about by a higher temperature, but the shift in the location of the chiasmata is very marked, and could only have been brought about by terminalization. A comparison of the short- with the long-chromosome cells (both grown at 40° C. for 2 weeks) reveals that a reduction in chiasma number as well as in a change in position has occurred, if one can assume here that the initial chiasma formation at any one temperature is a fairly constant phenomenon, an assumption that is upheld by genetic data in other organisms. The long-chromosome cells resulting from exposure to 40° C. for only 24 hours do not have a statistically significant increase in chiasma frequency

TABLE 2

DATA ON MAJOR COILS AND CHIASMA FREQUENCIES AT HIGH (39–42° C.) AND LOW (3.5° C.) TEMPERATURES

Temp.	Major coils per chromosome	Terminal chiasmata per cell	Interstitial chiasmata per cell	Total chiasmata per cell	Univalents per cell
42° C.	4.48	3.96	1.00	4.96	4.07
41° C.	4.70	0.80	0.06	0.86	10.26
42° C.	5.00	3.87	1.43	5.30	4.09
41° C.	5.44	5.2	0.64	5.84	4.90
42° C.	5.77	5.84	2.16	8.00	0.20
40° C.	6.00	6.78	3.03	9.81	0.77
39° C.	6.20	6.18	3.56	9.74	0.54
3.5° C.	6.00,	6.12	3.76	9.88	1.12
3.5° C.	8.14	6.00	5.80	11.80	0.37
3.5° C.	8.58	5.08	5.84	10.92	0.36

over the control cells, and this, it is believed, is due to the fact that chiasma formation occurred before the heat treatment was given, and hence only the position of the chiasmata has been affected.

That the development of the major coils affects terminalization is further substantiated by data in table 2. At 39–42° C. and at 3.5° C., where some variation was found in the coiling process, the cells showing the least number of coils possessed, in general, the least number of total chiasmata, the greatest proportion of chiasmata at terminal loci, and the greatest number of univalents. As the major coils progress in development, the chiasmata shift to more terminal loci, and those at the extreme ends of the chromosome arms may be pushed off and lost, as Moffett (1932) has shown occurs normally in Anemone.

Interesting in this respect are the observations of Upcott's (1937) in male-sterile Lathyrus. She finds that while no univalents were observed at diplotene and diakinesis, a number occurred at metaphase in this same plant. Chromosomes, therefore, which were paired in earlier stages become univalents at metaphase by a loss of chiasmata, although, it must be mentioned, this is not Upcott's interpretation, since she states that the completeness of pachytene pairing must vary during the life of the plant, a condition, however, which she assumes but did not observe. The difference in terminalization and spiralization between the normal and the mutant types Upcott found to be due to a greater time interval which the mutant has for prophase processes, this increased time relationship permitting chromosome contraction to proceed further, and also, according to Upcott and the electrostatic theory of terminalization, permitting repulsion forces to act longer. If, however, one looks upon terminalization as a function of the coiling mechanism, and therefore a more or less passive phenomenon dependent upon a parallel temporal process, then the terminalization of chiasmata becomes readily explicable without invoking

the presence of intangible forces necessary to bring about such a change.

It is known that the meiotic coiling cycle of Tradescantia is a continuous and progressive phenomenon (Swanson, 1941). The incipient coils are small and numerous, and as prophase proceeds, despiralization occurs with an increase in the diameter of the coil gyre together with a concomitant decrease in the number of coils. The progression is therefore from a long, laxly coiled thread to one which becomes relatively shorter and more rigid.[2] This may be readily seen by a comparison of prophase stages. The development of the minor as well as the major coils accentuates the shortness and the rigidity of the chromosomes. Under such conditions of increasing rigidity, the homologous chromosomes, bound to each other by chiasmata at the points of exchange, will tend to seek a spatial disposition that will most effectively distribute the force developed by the rigidity over the greatest possible area. As the rigidity increases, the regions between adjacent chiasmata will bow out and assume the form of loops which later come to lie at right angles to each other as in metaphase bivalents. In this respect the chromosomes may be likened to coiled wire springs. If two such springs were bound together by a series of exchanges, simulating chiasmata, identical configurations would result. Looked at from this point of view, the force induced by the development of the coils will eventually become greater than the force holding the chiasma at the point of exchange, and the chiasma will slide or be pushed along. In other words, the rigidity of the coiled system can be lessened by increasing the diameter of the loop between adjacent chiasmata, and an increase in diameter occurs when chiasmata move. Since

[2] It is assumed here that the chromonema, like any thread-like structure, will develop a certain rigidity as it becomes coiled and confined into an increasingly shorter space, the boundaries of this space being the walls of the matrix. This rigidity is also demonstrated somatically by the coiling of the sister chromatids in mitosis, these tending to straighten out and undo the relational twists as they develop somatic coils.

despiralization is a continuous process, with chromosome rigidity probably increasing proportionately, the chiasmata will continue moving after once started. This does not imply that all the chiasmata in a chromosome will move simultaneously, or over the same distance, for the degree of movement will be a function of the distance between adjacent chiasmata, other things being equal.

One is still confronted with an awkward dilemma, however, for any terminalization hypothesis must take into account the fact that most detectable chiasma movement is in a distal direction.[3] This fact forms one of the strongest supports of the electrostatic theory, since, according to this theory the centromere, by virtue of its special repelling force, would tend to move chiasmata away from it, *i.e.,* in a distal direction. However, a logical argument in favor of the despiralization hypothesis of terminalization can be advanced. In the first place, it should be pointed out that the greater size of the attachment loop need not be the result of a strong centromere repulsion, but is more probably a direct result of the fact that the distance from the centromere to the first proximal chiasma is generally greater than the distance between succeeding chiasmata. Or, as Mather (1940) expresses it, the differential distance is greater than the interference distance, and the most proximal chiasma reflects the probable location of original chiasma formation for that region. If the attachment loop is greater in size than adjacent loops, then on the basis of chromosome rigidity presented above, one might expect a slight movement of chiasmata proximally since the rigidity of the attachment loop would be less than that of the smaller adjacent loops, assuming, of course, that coiling along the chromosome is the same in all regions. This proximal movement, if present, could be detected only with difficulty, and would in any event soon cease, for a proximal movement would induce a greater rigidity by lessening the distance between adjacent chiasmata. The only direc-

[3] Trillium is thought to be an exception.

tion in which a constant reduction in the strain imposed by the coiled threads could be brought about would be a distal one, and once movement began it would presumably continue in the same direction for the initial inertia of starting the movement would have been overcome. Where the differential and interference distances are equal, the rigidity of the loops would be equal, and again, movement must occur at a distal point first before there can be any movement of proximal chiasmata. This offers ready support for Mather's contention that unless terminalization is complete only the most distal chiasmata are involved in movement. Loops will therefore increase in size at the expense of open arms until the chiasmata are terminalized. Loss of the chiasmata would occur only if the force developed were great enough to push the most terminal ones off the ends of the chromosome arms.

The data in Table 1 support the above hypothesis. It has been pointed out that the interstitial chiasmata in the long-chromosome cells are largely subterminal in position (Fig. 5) with only a few located in the region of the centromere. It is these latter chiasmata which, under normal conditions, remain interstitial at metaphase (compare the long-chromosome cells (40° C. for 1 day) with the control cells), while the other chiasmata are terminalized or become lost, as indicated by the data on the short-chromosome cells (40° C. for 1 day). Normal terminalization in *Tradescantia* therefore involves only the more or less distal chiasmata, with little or no loss of chiasmata, the change in ratio of interstitial in terminal chiasmata reflecting this movement. Also, in the long-chromosome cells, where there is only one chiasma per arm, the chiasmata are usually intersititial, whereas arms with two usually show one to be terminal. The force developed by the rigidity of the chromosomes, under such conditions of reduced despiralization, is insufficient to terminalize the single chiasma on either side of the centromere, but when an acentric loop is formed, the distal chiasma is forced to the end of the arm. When

only one chiasma per bivalent is formed, only very rarely is this terminal (*i.e.,* in the long-chromosome cells), for, as is readily apparent, no force due to despiralization could develop, since at least two points of contact would be necessary.

The above proposed hypothesis does not explain why terminalization is more pronounced in interlocked bivalents. One must assume here that interlocking interferes with the normal rate of crossing-over at the locus where the threads are interlocked. If the interlocking were at a distal locus, then no chiasma would form between that point and the distal end, and the interlocking would be lost by metaphase, whereas if located near the centromere, chiasma formation could take place only at distal loci, leaving a shorter distance for the chiasma to move, giving the appearance of greater terminalization.

It remains now to account for those organisms in which no terminalization occurs, but in which coiling is most certainly present. This situation is found, for example, in *Fritillaria, Tulipa* and *Lilium,* and a survey reveals that, almost invariably, these organisms have large, not too greatly contracted, metaphase chromosomes. They do not present the compact, rigid appearance such as is found in the chromosomes of *Campanula, Primula sinensis,* normal *Matthiola incana,* or *Rhoeo,* where terminalization is complete, or nearly so. Movement of chiasmata, therefore, must not be possible until the force developed by the coiling system reaches a *threshold value,* this value being of sufficient magnitude to offset the force which holds the chiasma at its point of origin (Mather, 1940). Those organisms in which no terminalization occurs possess a coiling system incapable of reaching this minimal value before metaphase sets in. This is borne out in part by Darlington's observation (Darlington and Janaki-Ammal, 1931) that in *Fritillaria* the small fragment chromosomes show complete terminalization while the long chromosomes show none. The same condition, more or less, holds for fragment chromosomes in *Trades-*

cantia, and is to be expected since small chromosomes, tightly coiled, would possess a greater rigidity, relative to their length, than would a longer, similarly coiled chromosome.

Mather (1940) has recently published information that is of considerable interest in the light of this concept of a threshold value for chiasma movement. He states that terminalization is an all-or-none reaction; once movement has occurred the reaction generally goes on to completion. The data given here indicate that terminalization is a progressive affair, dependent on a progressive coiling cycle, but if Mather is correct and there are no transitions between complete terminalization and terminalization at all, then one might possibly classify organisms into two groups on the basis of meiotic coiling behavior: (1) those in which a threshold value for the despiralization force is not reached soon enough for movement to begin, or which only affects the more distal chiasmata, and (2) those in which this value is reached soon enough before metaphase for terminalization to be completed. Under normal conditions, *Tradescantia* falls into the first category, but by the use of heat treatments it is possible to effect a transition between the two classes. It is also possible that the force necessary to cause an actual reduction in chiasma number is considerably greater than that needed to bring about merely a displacement of the more distal chiasmata, and that only those organisms having complete terminalization are capable of developing this force through their marked contraction.

Summary

Through a study of the effect of thermal treatment on meiotic coiling in *Tradescantia,* it has been possible to approach the problem of chiasma terminalization from an experimental point of view, revealing that forces other than electrostatic are operative in effecting chiasma movement. This has led to the formulation of a despiralization hypothesis of terminalization, based on purely

mechanical considerations, which states that the degree of terminalization is directly related to the degree of despiralization once despiralization has reached a certain threshold value.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Voles, Mice, and Lemmings; Problems in Population Dynamics. By CHARLES ELTON. New York and London: Oxford University Press, 1942: 1–496, frontisp., figs. 1–22. $10.00.

IN the hands of one less adept than Elton in phraseology much of the great mass of data presented in this monumental 496-page book might be about as interesting reading as Webster's Dictionary. Those who have read his previous works will find here that same simple way of spicing up what might easily have been very dry reading. To students of vertebrate zoology and to ecologists in general the author of "Voles, Mice, and Lemmings" needs no introduction as Elton's works are standard references on their shelves.

The present volume has the distinction of being the first comprehensive treatment of animal populations. In it the author not only brings together the pertinent literature of all countries from Biblical times to the present, but also includes an exhaustive review of the important work that the staff of the Bureau of Animal Populations at Oxford has been doing under his directorship. In discussing the researches of his own corps of workers, Elton is careful to give credit to the proper individuals. He has further limited his discussions of unpublished results to his own studies, not using those of his coworkers. He thus exhibits an admirable trait.

The principal thesis throughout the book is the phenomenon of periodic fluctuations in numbers of animals. Epidemiology, naturally, is stressed in relation to the

crashes after the years of great abundance. Biological control of mouse plagues (plagues here used in the sense of great abundance of mice and not of animals carrying disease such as bubonic plague) is discussed at some length, with particular emphasis on the "Danysz virus" method developed in France in 1893. Elton's conclusions in regard to control are that attempts at destruction of the rodents usually are not initiated until the peak in abundance has been reached and a decrease through natural causes is to be expected whether or not control methods are applied. Thus has a bit of witchery crept into certain methods of plague control in the past.

From the heretofore unpublished records of the Hudson's Bay Company and the Moravian Mission, Elton has pieced together a general picture of conditions in Labrador and Ungava for the past one hundred years. Foxes, although not mentioned in the title of the book, receive more attention than do the voles for these two areas. One can not wade through the sea of documentary evidence (quotations from journals and reports) and study the numerous tables and graphs without being convinced of the reality of what Elton terms "cyclic" behavior in the population of foxes, voles, and lemmings. The period between peaks (or lows, whichever one chooses) is usually four years with three- and five-year intervals less commonly and two to six year periods on occasion. The raw data on which he bases his conclusions are given so that any doubting Thomases may check the figures and interpret them as they choose. The following passage, relating to one particular group of figures, expresses Elton's attitude throughout the work. "There is not very much direct reporting on the coloured fox (see Table 50), but what there is agrees in practically every instance with the fur returns. So the evidence is thereby slightly strengthened. Coming to analysis of the figures, we have to use one trick that seems legitimate, as a sort of smoothing to bring out the main trends. With the arctic fox peaks, we counted no differences of less than four skins.

For the coloured fox we make this ten. Both are arbitrary, and if they seem like cookery, there is at any rate no secrecy about the recipe." To a less critical mind than Elton's there might be much misinterpretation of figures based on fur returns, but it seems to me that he has thought of and realizes the possible errors involved and has been particularly cautious in attempting to eliminate as many as possible.

Some readers, the reviewer among them, would like to see more discussion about the basic biological principles involved in this phenomenon of pulsating populations, but here again Elton's super-caution comes into play. To date we do not possess sufficient information gained through critical long-term studies in the field and laboratory to do much more than guess at what the causal agents might be and Elton realizes this perhaps better than anyone else. After summing up the work of his own group at Oxford, he says "From all this we begin to see vole populations in dynamic terms: with numbers, rates of increase, life-curves, and movements interwoven into a pattern that ten or twenty years of further work may enable us to understand completely. When we have understood this pattern, we shall have learned new principles that will apply to many other populations of animals." On these grounds we may excuse the author for making the present volume chiefly an historical account and a catalogue of information on animal populations. This in itself is a great service. We now have a solid foundation on which to build. The type of structure will depend on the ingenuity and imagination, and above all the intimate cooperation of the architects throughout the various countries who are working on the plans.

Lest I leave the impression that the book is of value only to a select group of zoologists who happen to be interested in population dynamics, I should like to state briefly some of its broader aspects. To the student of zoogeography there is a fund of basic information on movements, dispersal, and of course on populations. The

evolutionist, especially the adherent of the natural selection theory, must indeed be impressed by the rapid turnover in vole and lemming populations. Many of these small rodents are annuals or at most biennials. If a selective agent is at work it has much material from which to draw. Elton gives an example of how selection has changed the composition of foxes in Labrador since commercial trapping was initiated there. To quote (p. 284) "We have seen that every fox has a price on its head, but one which varies with the colour of the coat. There is at any time a natural balance in the proportions of different inherited colour phases. In Labrador this balance has shifted since the beginning of commercial fox-catching. Although it is theoretically possible that the change is a natural one—evolution caught in the act—the fact that the relative intensity of change follows the relative values of the three colours is strong argument that man has been the chief influence, perhaps the only one. Whichever is true, there is laid before us the interesting spectacle of evolution and selection in operation."

A short summary at the end of the volume would have been greatly appreciated by those whose field of specialization is somewhat removed from that here presented.

WILLIAM HENRY BURT

On Growth and Form. By D'ARCY WENTWORTH THOMPSON. Revised and Enlarged Edition. Cambridge: University Press, and New York: Macmillan Co., 1942: 1–1116, figs. 1–554. $12.50.

A SCHOLAR of the old school, trained in the classics and mathematics as well as many phases of biology, D'Arcy Wentworth Thompson presented during World War I a philosophy of biology. His large treatise "On Growth and Form" went through the years between the wars too little used by the increasing horde of narrowing specialists. Somewhat disappointed, we sense, but still enthusiastic and keen on biological synthesis, the now venerable scientist offers to a world again rent by wars a greatly enlarged and revised edition of his *magnus opus*.

The section on rate of growth is particularly increased, but additions, totalling more than three hundred pages, amplify the discussions throughout the book.

The expressed aim of the author, in both editions, has been to show "that a certain mathematical aspect of morphology, to which the morphologist gives little heed, is interwoven with his problem, complementary to his descriptive task, and helpful, nay essential, to his proper study and comprehension of Growth and Form." The emphasis throughout is on the mathematical background of form and development, simple to complex. Digressions are made to explain the mathematics of other biological phenomena, for instance, flight, and the color pattern of zebras. The order and symmetry that pervade the organic world are the themes of D'Arcy Thompson's philosophy.

With encompassing and now very rare facility the author quotes from ancient, medieval and modern authorities of all lands and tongues. Quite understandably some very pertinent recent work, such as that of W. K. Gregory on the changing basic structure of vertebrates, is left unnoticed. The rapidly expanding field of relative growth is mentioned and dispensed with as unnecessary, since many growth curves follow the straight-line equation well enough. In support of this view, despite the fact that the idea is now generally discarded except as an approximation, Thompson cites Hecht's early conclusion that the growth of each part of a fish is a linear function of the total length. The relative growth theory has already contributed too much to morphology and evolution to be so quickly brushed aside.

The fact that Cartesian transformations in body form and other "geometrical analogies" are of fundamentally distinct types in different groups, impresses the author as evidence of a fundamental discontinuity between these groups. He rightly sees that herein lies no argument against the theory of evolutionary descent. The different systems of form tend rather to show that the higher

groups are not derived from one another, but each separately from simple beginnings. This conclusion I regard as one of the fundamental laws of phylogeny. The tree of evolution is really a shrub, with many stems rising from the base.

Problems of Aging. Biological and Medical Aspects. Second Edition. Edited by E. V. COWDRY. Baltimore: Williams and Wilkins Co., 1942: i–xxxvi, 1–936, figs. 1–129. $10.00.

A CONSCIENTIOUS reviewer might grow aged in an attempt to digest all the data in this great compendium. Thirty-seven authors, including a number of outstanding leaders in general biology, physiology, medicine, psychology and anthropology, have focused their thoughts on a problem that has always been a main concern to man—and woman. The first edition of this book, issued in 1939, went far to develop this field into an integrated branch of science. The large amount of research on aging thus engendered has called for the issuance after only three years of this new edition of Problems of Aging. The work is revised throughout and contains nine new chapters by eleven new contributors.

Aging in plants and non-human animals is treated in the first four chapters, by separate authors. CLARK WISSLER then offers an anthropologist's view on problems of human senility, and LOUIS I. DUBLIN presents a summary of statistical data on age in man. There follow fifteen chapters on aging of systems, six on the aging processes of tissues and six on the psychological, clinical and sociological aspects of the problem.

The most fundamental deficiency in the volume, from the biologist's viewpoint, is the lack of a thorough integration of ideas on the biological significance of aging. Why has it come about that organisms, of course including man, grow old and die? Why do some live but a few days, others for more than a century? Why do some kinds of organisms stop growing whereas others continue to grow indefinitely? Why do many organisms retain fecundity throughout life, while others become sterile?

How far is senility an unavoidable physicochemical consequence, and how far is the life-span regulated by natural selection? There is presented in the book under review much information bearing on these problems, particularly data from researches on mammals, but far too little space is given to the general biology of senescence.

Studies in the Genetics of Drosophila. II. Gene Variation and Evolution. Directed by J. T. PATTERSON. Univ. of Texas Publ., 4228, 1942: 1–200, 8 pls., 30 figs. $1.00.

TEN contributions, by J. T. PATTERSON and six associates, are brought together in this publication. They constitute a notable contribution to genetics, sex determination, speciation and systematics. Dr. PATTERSON first shows that the several interspecific crosses now known in *Drosophila,* yielding varied sex ratios, provide material for an analysis of the genetics of evolution. WILSON S. STONE deals with heterosis in crosses between different strains of *Drosophila hydei.* LINDA T. WHARTON analyzes the comparative morphology of the chromosomes in the *D. repleta* group, with particular reference to the problem of sexual isolation. J. F. CROW follows with an investigation of cross fertility and isolating mechanisms in the *D. mulleri* group. Studies are then reported on genetic relationships in the *D. melanica* and *D. funebris* groups, respectively, by A. B. GRIFFEN and G. B. MAINLAND. Intersexes produced by a dominant mutation in *D. virilis* are analyzed in detail by W. W. NEWBY. Then WILSON S. STONE takes up the Ix^B factor and sex determination. Dr. PATTERSON describes the distribution of the *virilis* group in the United States, and then, with two associates, gives a genetic and cytological analysis of the *D. virilis* group—a study of great speciational significance. A minor criticism might be directed against the use of the letter X in place of the multiplication sign in the names of hybrids.

Catalog of Human Crania in the United States National Museum Collections: Eskimo in General. By ALEŠ HRDLIČKA. Proc. U. S. Nat. Mus., 91, 1942: 169–429, fig. 39.

THROUGH the energy of the author more than 2,000

Eskimo crania have been amassed in the United States National Museum. This paper is essentially a presentation of detailed data taken from this rich material. Tables of measurements and simple indexes cover all but 16 pages. Statistics to indicate the reliability of averages and the similarity or divergence between groups are lacking. We must, therefore, either analyze the data or take it on faith that "the measurements show some local differences but a close basic similarity is evident throughout. Here, plainly, is a single physical strain of the human family, differing only, as any other large and widely scattered strain would, in secondary peculiarities." Whatever speciation the Eskimo has undergone seems certain, Hrdlička believes, to have taken place in Arctic Asia. In addition to the Eskimo, four groups of non-Eskimo people are said to inhabit the coast and islands of Alaska, and "it seems probable that no other larger groups will be discovered." "From place to place the Eskimo differs somewhat in stature and even in head form, but with one exception there is no possibility of subdividing them into distinct types." I suppose it is nice to be able to be positive.

Fading Trails. The Story of Endangered American Wildlife. Prepared by a Committee of the United States Department of the Interior. National Park Service. Fish and Wildlife Service. DANIEL B. BEARD, Chairman. FREDERICK C. LINCOLN. VICTOR H. CAHALANE. HARTLEY H. T. JACKSON. BEN H. THOMPSON. Illustrations by WALTER A. WEBER. Edited by CHARLES ELLIOTT. New York: Macmillan Co., 1942: i–xv, 1–279, 32 figs., 4 col. pls. $3.00.

Too many cooks have not spoiled this broth. They have concocted a dramatic story of the extermination and dangerous reduction in numbers of many of the vertebrate species of the United States and its territories. They have convincingly set forth the preservationist point of view that species are worth saving for their esthetic, educational and scientific value, even though they are of "no use" or even mildly harmful to man's interests.

As usual in wildlife matters, however, the treatment is woefully unbalanced. Mammals and birds are adequately considered; their interests were represented by the entire committee responsible for the volume. Although an effort was made to include the lower types of wildlife, only 34 pages were allowed for the chapters on fishes, reptiles and invertebrates; only five species of animals other than birds and mammals were considered; only commercially important species of the other groups were discussed; the point of view was changed from one of saving species from extinction to one of preventing serious depletion ("commercial extinction"). The "story of endangered American wildlife" could have been told very much better for the cold-blooded animals.

Comparative Vertebrate Anatomy. By LIBBIE HENRIETTA HYMAN. Chicago. University of Chicago Press, 1942: i–xx, 1–544, figs. 1–136. $3.50.

IN this volume Dr. Hyman's much-used Laboratory Manual for Comparative Anatomy metamorphoses into a full-fledged text. All who are acquainted with the author and her work—and that includes most American zoologists—have felt sure that this new treatise from her pen would be a superior product, excellent in detail, clarity, accuracy and originality. They have not been disappointed.

The text begins with General Considerations on Animal Form, with accounts of descriptive terms, planes and axes, symmetry, metamerism, cephalization and homology and analogy—altogether short and clear enough for the student to learn by heart. The second chapter, dealing with the Chordata in a general way, presents a classification based on Romer. The following chapters present the Essential Features of Lower Types, External Anatomy and Adaptive Radiation in Gnathostomes, and General Features of Chordate Development. Nine more sections deal with the systems, one by one.

Dr. Hyman's book may prove too detailed to serve as the sole text in any but the longer courses in comparative

anatomy, yet will hardly serve as a detailed reference book on chordate structures. Nevertheless it will and should find its place among the much-used books of many students and professional biologists.

The Copepods of the Plankton Gathered during the Last Cruise of the Carnegie. By CHARLES B. WILSON. *In:* Scientific Results of Cruise VII of the CARNEGIE during 1928–1929 under Command of Captain J. P. Ault, Biology—I. Carn. Inst. Wash. Publ. 536, 1942: i–v, 1–237, maps 1–2, charts 1–6, figs. 1–136. $2.50 (paper), $3.50 (cloth).

The Oceanic Tintinnoina of the Plankton Gathered during the Last Cruise of the Carnegie. By ARTHUR SCHACKLETON CAMPBELL. *In:* Scientific Results of Cruise VII of the CARNEGIE during 1928–1929 under Command of Captain J. P. Ault, Biology—II. *Ibidem,* 537, 1942: i–iv, 1–163, pl. 1, figs. 1–128. $1.50 (paper), $2.50 (cloth).

ONE of the most important of the many major, painstaking works of the late Charles Branch Wilson, the first paper in this series, we are assured by Waldo Schmitt, "for the first time in the history of oceanography gives the directly comparable results of simultaneous, three-level tows made in all oceans with identical gear, accompanied by full station data." It is therefore a major contribution to oceanography as well as carcinology. The Pacific plankton was found to be much richer than that of the Atlantic—confirming the results of studies on fishes and other groups. It is a source of gratification to see finally appearing the biological results of the last, ill-fated expedition of the research vessel CARNEGIE.

ALTHOUGH it is essentially a detailed systematic treatise, Campbell's study of pelagic protozoa leads to several generalizations. The lorica is a satisfactory basis for the taxonomy of the group, and "is the external expression of the internal peculiarity of the animal." The Tintinnoina are an important element in the pelagic plankton. They are distributed, with definite limitations, in accordance with five faunal regions: boreal, austral, tropical, coastal and temperate. The species occur within characteristic physical and chemical limits. Superposed stratification of faunas occurs.

NOTICES OF NEW BOOKS

The Biological Action of the Vitamins. ***A Symposium.*** Edited by E. A. EVANS, JR. Chicago: University of Chicago Press, 1942: i–viii, 1–227, 32 figs. $3.00.—Fourteen contributions by fifteen authorities make up this volume, which is one of the products of the Fiftieth Anniversary celebration of the University of Chicago. There is hardly a field of biology in which a knowledge of the effects of the vitamins does not impinge. This coordinated résumé of our knowledge on the subject should therefore have wide use and value.

A Manual of Experimental Embryology. By VIKTOR HAMBURGER. Chicago: University of Chicago Press, 1942: i–xvii, 1–213, figs. 1–45c. $2.50.—This simplified text consists of five parts: 1, Equipment and Instruments; 2, Experiments on Amphibian Embryos; 3, Experiments on the Chick Embryo; 4, Regeneration (Reconstitution); and 5, The Gradient Theory. The last two sections utilize planarians chiefly; otherwise only amphibians and the chick are used. It is to be hoped that, with a good text on experimental embryology available, more departments will break with the tradition of restricting the zoology student's training in embryology to a conventional premedical course.

Medical Parasitology. By JAMES T. CULBERTSON. New York: Columbia University Press, 1942: i–xii, 1–285, pls. 1–21, figs. 1–16. $4.25.—Increases in the rate and amount of global transportation and travel have augmented the danger of spreading diseases caused by animal parasites. Medical students and practitioners therefore stand in greater need than ever before, of being trained in the diagnosis and treatment of such diseases. "Medical Parasitology" has been prepared to help fill this need, and appears well adapted to that end. The general biology of animal parasitism receives some attention, in the chapters on infection (including a discussion of adaptations of parasites for infecting their hosts); on epidemiology; and on natural resistance and acquired immunity, subjects treated more fully in the author's recent "Immunity Against Animal Parasites."

College Zoology. Fifth Edition. By ROBERT W. HEGNER. New York: Macmillan Co., 1942: i–xvii, 1–817, col. pls. 1–8, figs. 1–441. $3.75.—Many zoologists have had the good fortune to be brought up on this text, and others would have been more soundly trained on such a foundation. The work is too well known to call for a detailed review in a biological publication. The fifth, posthumous edition strikes a desirable balance between a systematic and a topical approach. The treatment has been clarified by many new figures, including eight magnificent colored plates by the distinguished animal artist, R. Bruce Horsfall.

A General Science Workbook. By DOUGLAS ELLIOTT WEIDNER. Lancaster, Pa.: Jaques Cattell Press, 1942: 1–215, many figs. $1.00.—Designed for use in a two-semester junior high school course in general science, this workbook is neatly illustrated, save for a hardly recognizable outline map of the United States.

The Seashore Parade. By MURIEL LEWIN GUBERLET. Lancaster, Pa.: Jaques Cattell Press, 1942: 1–197, 58 figs. and 6 col. pls. (by JAN OGDEN). $1.75.—This is a good biology of the seashore, written down to the level of small—very small—boys and girls. It should go far toward instilling an interest in biology among children living on or visiting the coast. It is accurate so far as it goes, and is largely devoid of the sentimental goo that commonly goes into children's books on animals.

Contribuciones a la geogotánica ecuatoriana—Anotaciones sobre la vegetación del norte de Quito: desde Cotocollao y San Antonio hasta el Rio Guayllabamba. By M. ACOSTA SOLIS. Quito: Imp. de la Universidad, 1942: 1–120, figs. 1–44 (reprint from Anales, Univ. Centr. Ecuador, 67, 1942: 5–83, figs. 1–44).—In this book the Director del Instituto Ecuatoriana de Ciencias Naturales deals with the "geobotany" (geography, climatology, vegetation, agriculture, and other features) of a portion of his country. It is an important contribution toward an understanding of the ecology of South America.

We have also been favored with a copy of "Flora" (Instituto de Ciencias Naturales, Quito, Ecuador), Epoca 2a, vol. 2, nos. 3–4, May, 1942 (192 pp.). This issue contains a number of important articles on the vegetation of Ecuador.

Geographical Narrative and Cartography. By DOUGLAS MAWSON. *In:* Australian Antarctic Expedition, 1911–14, under the Leadership of Sir Douglas Mawson, Sci. Repts., (A) 1: 1–364, figs. 1–30, pls. 1–124, 11 map pls. 3£, 17s., 6d.—In the midst of the second war which has intervened since the explorations of the Australian Antarctic Expedition, the Australian Government sees fit to issue this monumental volume, and is to be complimented for so doing. The major contribution—a very large one—is to Antarctic geography, but through it all runs a thread of stirring adventure by stout-hearted men. Included are numerous biological observations, particularly on birds and mammals.

SHORTER ARTICLES AND DISCUSSION

MOSAIC SALIVARY GLANDS IN SCIARA

SPECIAL interest attaches to mosaic salivary glands of Diptera because the enormous salivary gland chromosomes reveal details of antecedent chromosome behavior which could not be detected in tissues possessing ordinary mitotic type chromosomes. Likewise, such glands may be used to throw light on cell lineage and on the behavior of cells and nuclei during embryonic and larval development. Among the cases of mosaic glands described by other authors, the following appear to be the most significant in the present connection. L. V. Morgan (1939) found a gland in *Drosophila melanogaster* in which two (possibly three) cells exhibit a translocation not present in the other cells. This case shows that translocations may occur spontaneously in this material during embryonic or larval development. The distribution of the cells in the gland is not given. Kaufmann (1939) has reported a case in *Drosophila melanogaster* in which "some of the nuclei of a pair of glands showed a duplication in the form of a reverse repeat," whereas others were deficient for this chromosome region. Since in this case the father was x-rayed, it seems probable that the chromosome in question was longitudinally double in the sperm at the time of irradiation and that both strands were recovered. Likewise, the evidence indicates that both daughter nuclei from the first cleavage contributed descendants to the salivary glands (see Helfer, 1940). No detailed evidence concerning cell lineage is given. Helfer (1940) has described two cases in *Drosophila pseudoobscura,* likewise from irradiation experiments. In each case both glands of the pair were mosaic, and in one case four types of nuclei were found. The latter case appears to lend support to the view that a delay of one or more cell generations may occur between the time of treatment with x-rays and the occurrence of the resulting chromosome aberrations. Here again, the problem of cell lineage is not specifically considered.

The present account deals with a mosaic found in a species hybrid in Sciara. The glands under consideration were taken from a female larva obtained in a cross between a female of *Sciara ocellaris* Comst. and a male of *S. reynoldsi* Metz (see Metz and Lawrence, 1938). The two glands from this specimen were pre-

pared in the usual manner (aceto-carmine smear), using only the broad part of each gland, which is the part most favorable for chromosome study (Fig. 1). The two glands are well separated

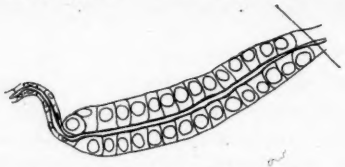

FIG. 1. Outline drawing of the thick basal portion of a salivary gland from a larva of *Sciara coprophila*. The gland is flat and consists of two rows of cells, separated by the duct.

on the slide and quite distinct from one another. Although somewhat disorganized in smearing, they are sufficiently intact to permit a partial reconstruction of the original positional relationships of the cells. One gland is composed entirely of cells having the expected hybrid chromosome group, and hence is of no special interest in the present connection. The other, however, is a mosaic having the following constitution:

Out of a total of 29 cells, 16 are of the expected hybrid type, 12 are pure *ocellaris* in chromosome constitution and one has the hybrid group plus an extra chromosome B of *ocellaris*. The distribution of these cells is shown in Fig. 2, where the hybrid cells

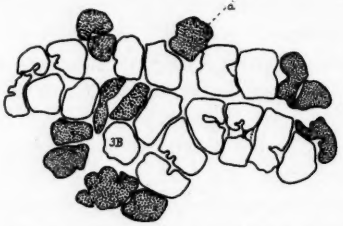

FIG. 2. Diagram of the mosaic hybrid gland shown in Fig. 3. Hybrid cells are shown in outline and pure *S. ocellaris* cells are stippled. The cell marked *3B* has an extra chromosome B of *S. ocellaris*.

are drawn in outline, the pure *ocellaris* cells are stippled and the cell having the extra *ocellaris* B chromosome is marked *3B*. A photomicrograph of the gland is shown in Fig. 3. Before considering the significance of the distribution of these cell types, attention will be given to the origin and special characteristics of the unexpected chromosome groups.

The simplest case is that of the single cell having the one extra chromosome. The presence of an extra chromosome here is inferred from the fact that the *ocellaris* chromosome B in this cell is fully twice as large (thick) as its mate from *reynoldsi* or any of the other single chromosomes. This is shown in Fig. 4. The *ocellaris* B has the proportions and staining qualities of the chromosomes (pairs) found in normal cells of *S. ocellaris,* and is like those found in the pure *ocellaris* chromosome groups considered below. So far as I have been able to observe it is not visibly differentiated into two elements, but it presumably came from a

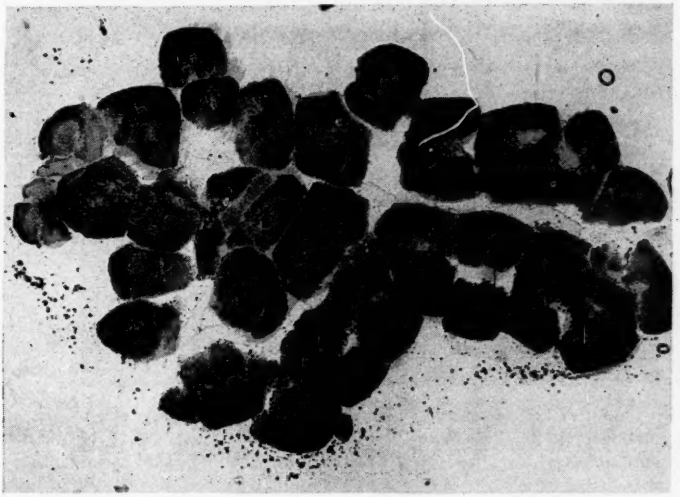

FIG. 3. Photomicrograph of the hybrid gland shown in Fig. 2. From aceto-carmine smear. Slide 457 3. ×50.

doubling of the original *ocellaris* member in an ancestral cell. There can be little doubt that this condition arose through simple non-disjunction of the *ocellaris* chromosome B, such that the two sister halves went to the same pole at some mitosis prior to formation of the definitive salivary gland cells. Since only one cell of this kind is present and it is near the middle of the gland (broad part), and since the reciprocal type, lacking an *ocellaris* chromosome B, is not present, it is to be inferred that probably the non-disjunction took place shortly before the last mitosis and that the deficient cell either degenerated or did not happen to be included in the gland.

In considering the twelve cells which possess the pure *ocellaris* chromosome group it may be recalled that the salivary gland chromosomes of *Sciara ocellaris* are sufficiently different from those of *S. reynoldsi* so that there is no difficulty in distinguishing between them (see Metz and Lawrence, *l.c.;* Metz, 1938, and Metz, 1941). Detailed examination of the twelve cells has shown that in four of them all four chromosomes are clear, in four others all but one chromosome are clear, and in the remaining four two are clear. In all cases the chromosomes which are clear are *ocellaris,* and there is no evidence of the presence of a *reynoldsi* chromosome in any of the groups. It is inferred, therefore, that the groups in question possess only *ocellaris* chromo-

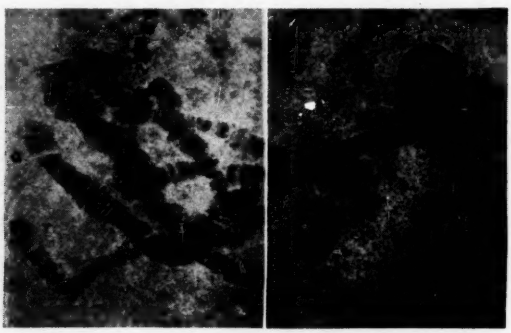

FIG. 4. Photomicrograph of portion of the hybrid nucleus (*3B* in Fig. 2) having an extra chromosome B of *S. ocellaris. o,* the double *ocellaris* B chromosome; *r,* the single *reynoldsi* B chromosome. The right and left halves of the figure are photographed at slightly different focal levels. × 750.

somes. The size and proportions of these chromosomes are those characteristic of the chromosomes (pairs) found in the salivary glands of ordinary pure *ocellaris* larvae, rather than of the single chromosomes in the hybrid nuclei. This indicates that the original *ocellaris* chromosomes from which these were derived either increased in size to conform to the size of the cells or became doubled by ordinary division. Biparental origin in this case is out of the question.

The chromosomes under consideration might conceivably have been derived originally from a polar body nucleus or from one daughter of a precociously divided egg nucleus (germinal vesicle), but this seems improbable both on general grounds and

because of the presence of the aberrant nuclei in only one gland. A more plausible alternative is that the obs rved condition arose through doubling or enlargement of the original *ocellaris* chromosomes, accompanied by loss or complete segregation of the *reynoldsi* chromosomes. This modification could have come about in either of two ways (omitting one or two possibilities which seem too remote for consideration). One is by complete nondisjunction of the chromosomes, such that the split *ocellaris* chromosomes all went to one pole and the *reynoldsi* chromosomes to the other. Complete separation of maternal from paternal chromosomes would not be as surprising in Sciara as in other organisms because at spermatogenesis a process of selective segregation is known to occur normally in this group (for review see Metz, 1938). This interpretation is also supported by the apparent case of non-disjunction of chromosome B considered above. Such a process should give rise to corresponding pure *reynoldsi* cells. Since no cells of this latter type were found it would be assumed that they either degenerated or were not included in the tissue studied. Neither fate would seem surprising. The *reynoldsi* chromosomes alone might well fail to function properly in *ocellaris* cytoplasm, thus leading to death of the cell. Or, if the nondisjunction occurred early, the two daughter nuclei might have separated widely and only one contributed descendants to the salivary gland tissue. The second possibility is that the *reynoldsi* chromosomes, being of paternal origin, were eliminated by a process comparable to that which regularly eliminates one or both paternal X chromosomes during cleavage in Sciara (DuBois, 1933; Metz, 1938), and that the remaining *ocellaris* chromosomes became doubled or enlarged. Such a view is favored by the absence of pure *reynoldsi* cells, but is considered improbable because of the difficulty of accounting for the required doubling or enlargement of the chromosomes.

The last feature of interest concerns the bearing of the phenomena on cell lineage and on the origin of the salivary gland cells. Although not critical, the evidence, like that of Helfer (*l.c.*), weighs strongly in favor of the view that chance position and not cell lineage determines which cells shall take part in the formation of the glands. The following considerations all seem to bear on this point:

(1) If a salivary gland were made up of a homogeneous cluster of cells, all derived from a primordial mother cell, the hybrid

cell having an extra chromosome B should be accompanied by an adjacent cell lacking this chromosome, which is almost certainly not the case. The presence of this single trisomic cell seems best explained on the assumption that the non-disjunction occurred at an earlier cell division and that the other products of that division did not contribute to the formation of the gland. This observation is also in harmony with the finding of E. Kirkwood (unpublished) that in Sciara no cell division occurs after the salivary gland is first differentiated.

(2) The distribution of the twelve pure *ocellaris* cells has a similar implication if, as seems probable, all twelve owe their origin to modification of a single ancestral hybrid cell. That the latter mode of origin is correct is indicated by three considerations. (*a*) The rare occurrence of such a modification, as indicated in turn by the fact that in our extensive studies of hybrid salivary glands no other case like the present has been found. It is improbable that such a rare phenomenon would occur two or more times in the cells giving rise to one gland. (*b*) The absence of such modified cells in the other gland. If the modification occurred two or more times it would be surprising to have the products limited to one gland. (*c*) The absence of the complementary pure *reynoldsi* cells, or their remains. This feature is best explained by assuming a single modification, relatively early in development, followed by loss of the *reynoldsi* nucleus or by wide separation of the daughter nuclei such that only one contributed descendants to the salivary gland.

Although the architecture of the gland has been disturbed in making the smear, part of the organ is intact and it is evident that the twelve modified cells are not contiguous. Assuming a common origin for these cells, therefore, it follows that they have become scattered during development and that their presence in the gland is probably due to their being included by chance in the region of the ectoderm which ultimately gave rise to gland tissue. If this is correct it is possible to estimate approximately when the modification occurred.

At the right end of the gland, as shown in Fig. 2, there are three pure *ocellaris* cells. To the left of these the cells are hybrids for some distance. Whether the single *ocellaris* cell at the top (*a*, Fig. 1) belongs in its present position or not is uncertain. To the left of this the *ocellaris* cells all lie in pairs, but the pairs are not together. Possibly they formed a single cluster in the

intact gland, but it is difficult to see how simple pressure in smearing could have given them their present positions if that were the case. Presumably each pair represents two sister cells. As a whole, therefore, the evidence points to the conclusion that the disturbance giving rise to these cells occurred only a few cell generations previous to the final mitoses, but early enough to permit dissemination of the modified cells among the unmodified ones. Presumably it did not occur in earliest cleavage stages because no modified cells are found in the other gland. The latter inference is based on Helfer's findings in Drosophila. In Helfer's (*l.c.*) cases, both glands of the pair were mosaics of the same kinds of cells, which indicates that both products of the first cleavage nucleus, at least, contribute descendants to each salivary gland. If this is true of the first cleavage nucleus it is probably also true of other early ones.

It seems probable that the phenomenon which gave rise to the pure *ocellaris* chromosome groups in the present case reflects a relatively unstable condition in the species hybrid and would occur very rarely, if at all, within a pure species of Sciara.

Summary

A mosaic salivary gland was found in a larva derived from a cross between *Sciara ocellaris* (♀) and *S. reynoldsi* (♂). The other gland of the pair was entirely hybrid in constitution as expected.

Of the 29 cells in the portion of the gland used in making the preparation, 16 are of the expected type, 12 have the chromosome group typical of pure *ocellaris* without any *reynoldsi* chromosomes, and 1 has the ordinary hybrid group plus an extra *ocellaris* chromosome B.

It is inferred that the last-mentioned condition arose by a simple non-disjunction of the *ocellaris* chromosome B in an ancestral embryonic cell.

The pure *ocellaris* cells seem best accounted for on the assumption that through complete non-disjunction (selective somatic segregation) or selective elimination, in one ancestral cell, the *ocellaris* chromosomes have been separated from the *reynoldsi* chromosomes and have been doubled. Nuclei containing the *reynoldsi* chromosomes presumably degenerated or did not happen to be included in the salivary glands.

Assuming that the original modification occurred in only one

cell it is inferred from the distribution of the cells that the change took place during late cleavage and that the cellular constitution of the salivary glands in Sciara is not predetermined by cell lineage, but depends on the chance position of the cells in the early embryonic tissue (ectoderm).

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THE POLYMORPH MUTANT OF *DROSOPHILA MELANOGASTER*

THE present note will be devoted to a brief characterization of a *Drosophila melanogaster* mutation which so far as the author knows is unique in the great diversity of the phenotypic effects which it evokes. The mutation has occurred at least twice in the course of Drosophila work, having been detected once by Mr. V. Bryson in October, 1939, and once by the present author in February, 1941. The latter occurrence was during a study of a case of high spontaneous mutation frequency (Neel, in press). In both instances it was found in an inbred Oregon wild-type strain; the two Oregon strains involved probably had a common origin in 1926. The mutation is a recessive, located on the second chromosome, and has been named polymorph (*pym*) by Bryson (1940). Salivary gland cell chromosomes appear normal, although until the mutation has been more definitely localized the possibility of a small aberration can not be excluded (P. N. Bridges, J. Lane). The present study is based upon the second of the two alleles which have been found.

Mutant flies of both sexes are completely sterile. Since its dis-

covery a stock of the mutation has been maintained by making up, each generation, 9–10 single pair matings of phenotypically normal flies from a culture segregating for *pym* which in turn was the result of a similar single pair mating. Four ninths of such cultures are expected to segregate for the mutant. While this was a somewhat laborious method of maintaining the stock, it made possible an accurate appraisal of gene effects, since all the flies in a culture segregating for *pym* were genetically very similar except for the *pym* locus. In addition, a balanced *Cy/pym* line was used in some of the viability tests to be reported below.

Among the readily observable effects of the mutation are the following: *Eye color:* The eye is a translucent ruby in color. In alcoholic solution it decolorizes rapidly, becoming white with a pale pink center. The ocelli are slightly paler than in wild-type flies. *Body proportions:* The humeral angle is more prominent and bulging than in the wild-type, while the scutellum is relatively long and pointed. Thus, in 31 *pym* males the ratio of scutellar length to total thoracic length was 0.314 ± 0.0029, while in 37 phenotypically normal males from the same cultures the ratio was 0.293 ± 0.0020. The tibia, particularly of the hind leg, is often bent or bowed. The femur may be deformed to a lesser extent. The haltere is enlarged. *Wings:* The wings of *pym* flies are small, reaching only to the tip of the abdomen, and appear thin-textured and often wavy-surfaced. In *ca.* 5 per cent. of the flies they fail to expand fully. In alcoholic solution the two lamina composing the wing often separate. In 90 per cent. of the wings examined the second cross-vein was interrupted or missing. More rarely (*ca.* 2 per cent.) the first cross-vein is also abnormal. Plexuses of extra venation are often present, near the tip of the wing or in the third posterior cell. These may appear as diverticula off one of the normal veins or lie free in the wing cell. The marginal hairs of the wing are irregular. Shallow incisions may be present in the posterior margin of the wing. *Chaetae:* The frequency of occurrence of bristles at 20 standard loci was checked in 146 *pym* males and 158 phenotypically normal males from the same cultures. The non-*pym* flies showed an occasional missing or extra bristle, but none of the departures from the wild-type pattern was significant. *pym* flies, on the other hand, showed significant departures from the normal segregants of the same cultures at three bristle loci. The mean

frequency of occurrence of the ocellar bristle was 0.945 ± 0.0189 (controls -1.000), of the postvertical bristle, 1.041 ± 0.0191 (controls -0.987 ± 0.00889), and of the humerals, 2.096 ± 0.0244 (controls -1.981 ± 0.0167). The *pym* mutation thus both adds and removes bristles. Moreover, all bristles in *pym* flies are more slender than in wild-type, although of normal length. The one known exception to this statement is the anterior dorsocentral bristle, which is shorter than normal. The acrostical hairs are less regularly arranged in rows in *pym* than in wild-type, but the total number is about the same in the two phenotypes. The hairs on the dorsum of the head, particularly the ocellar, are also disarranged. *Reproductive apparatus:* The external genitalia are normal in both sexes. However, the internal genitalia are markedly imperfect, more so in the female than in the male. In the latter the testis is small, semi-transparent and less coiled than usual; the paragonia are also small. There is considerable variation in the extent of these abnormalities. The females have very small and rudimentary ovaries, but normally developed spermathecae. At the same time, and perhaps as a corollary of this, a more abundant fat body is present. *Body weight: pym* flies are smaller than wild-type. The mean weight of 46 *pym* males which were collected during the first three days that a series of bottles yielded adults and which were given unlimited access to food prior to weighing was 0.718 mg. The average weight of 100 wild-type males emerging from the same bottles during this period and similarly treated was 0.792 mg. *Viability:* As might be expected in view of the manifold effects of *pym,* the viability of the mutant is poor. In 20 cultures of a *pym*/+ × *pym*/+ cross, the total ratio of wild-type to mutant flies was 1,392 to 66, instead of the expected 1,093.5 to 364.5. The viability of *pym* was thus .18 normal. In 17 bottles of a balanced *Cy/pym* stock the ratio of *Cy/pym* to *pym/pym* was 2,057 to 169, instead of the expected 1,484 to 742, giving a viability of .23 for the mutant. The viability of the two sexes is not significantly different. *Time of emergence:* In order to test the effect of *pym* on rate of development, the offspring of 10 single pair matings of *pym*/+ flies were counted at two-day intervals over an eight-day period, beginning two days after the first flies emerged. The per cent. of *pym* flies appearing in successive counts was as follows: (the figures in parentheses indicate the number of flies on which the per cent. is based): first, 4.65 ± 1.21 (301); second, 5.30 ± 1.95 (132);

third, 10.53 ± 3.52 (76); fourth, 9.43 ± 4.01 (53). While this is suggestive of a retardation, in 17 segregating *Cy/pym* bottles counted in the same way the results were: first, 5.23 ± 1.04 (459); second, 8.80 ± 1.36 (432); third, 5.26 ± 0.89 (627); fourth, 10.45 ± 1.15 (708). This is a more ambiguous result. Although there may be a slight retardation, it is certainly much less than that known to be associated with many other phenotypically less striking mutants.

For ...er variety of effects *pym* is probably unique among spontaneous *D. melanogaster* mutations. Other well-known examples of less extreme spontaneous pleiotropic mutants in this species are spineless-aristopedia (Sturtevant, 1929, see also DIS–9), Stubble (Dobzhansky, 1930), short wing (Eker, 1939) and engrailed (Brasted, 1941), to mention only a few of the more completely described. Among the numerous mutants discovered in other Drosophila species ocelliless in *D. funebris* (Spencer, 1928) and engrailed in *D. hydei* (Spencer, 1942) are outstanding instances of factors with manifold effects. Similar examples could be quoted from other plant and animal species. In such cases as these, where the diversity of effects on embryologically unrelated structures is so great, it is difficult to conceive of mutation and multiple allelomorphic series in terms of simple quantitative, or even qualitative, variations in the amount of a single, gene-controlled enzyme, unless the same enzyme may play a rôle in a variety of different enzyme systems. This seems unlikely from what is known of enzyme specificity. Rather, several distinctly different primary effects of a single mutation, and hence of a single gene, are indicated. We are thus led to the well-known hypothesis of gene structure whereby some—not necessarily all—genes are regarded as compound but completely linked systems of actually or potentially independent variables (*cf.* Silow, 1939). As an alternative to such a concept we have to postulate that the pleiotropic mutant is really the result of the simultaneous loss or inactivation of several different closely linked factors. This explanation is improbable in the present case, since (1) the mutation is recessive, (2) salivary gland cell chromosomes appear normal, and (3) the same mutation has occurred at least twice. Such an explanation is also improbable in cases where a pleiomorphic mutant of one species has a homologue in another species, such as the spineless-aristapedia mutants of *D.*

melanogaster and *D. simulans* (Sturtevant, 1929) or the engrailed mutants of *D. melanogaster* and *D. hydei* (Spencer, 1942).

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A SECOND OCCURRENCE OF THE CHARACTER PINK-EYE IN THE DEER MOUSE

THE occurrence of an abnormal color variation in a wild population is rare enough to be of interest. The appearance of the same heritable character in unrelated stocks also is an uncommon event. It seems desirable therefore to put on record a case which presented both these phenomena.

A peculiarly colored male deer mouse (*Peromyscus maniculatus artemisiae*) was trapped in the spring of 1941 by Glen Brown in Ravalli County, Montana, along a ridge on the west side of the river, near highway 93, about due west of Como School House, which is about eight miles south of Hamilton. No other specimens like it were seen in this region, although a number of specimens of *Peromyscus* were trapped. The exceptional individual was given to Dr. William L. Jellison, of the Rocky Mountain Laboratory, who kindly shipped it to the University of Michigan and supplied the trapping record.

In color this mouse closely resembles a variation found by Dr.

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F. B. Sumner (1917) in cage-bred hybrids between the subspecies *rubidus* and *sonoriensis* from California, and shown by him to be inherited as a single-factor recessive character (1917 and 1918). This trait was originally described by Sumner as "partial albino" (1917); its designation was later changed by him to "pallid" (1918); and F. H. Clark (1936) applied the name "pink-eye" to it because of its similarity both in appearance and linkage relations to the character of this name in the Norwegian rat and house mouse.

Tests were made to determine whether the character in field-caught *artemisiae* had a genetic basis similar to that in the pink-eye mice of California origin. Matings between this mouse and five wild-type females produced twenty offspring, all of which were wild-type in appearance. In an F_2 generation of twenty-six individuals seventeen were wild-type and nine showed the pink-eye characteristics. This result indicates that the color variation in the mouse from Montana is inherited as a recessive trait. A pink-eye female derived from the *sonoriensis-rubidus* hybrids was then mated to the wild-caught *artemisiae* of similar color. From this cross nine offspring were obtained in the first and seventeen in the second generation. All these mice were pink-eyes. The character which appeared in the two widely separated stocks must therefore be determined by genes at the same locus.

Variation in shade occurs among the pink-eye mice derived from the *sonoriensis-rubidus* hybrids (Sumner and Collins, 1922). The shade of the pink-eye *artemisiae* lies well within the range of variation shown by the other stock. The possibility exists, however, that some undetected difference exists between the potentialities of the pink-eye gene from *artemisiae* and those of the pink-eye gene from *sonoriensis* or *rubidus*. It is therefore impossible to say whether the genes from the two sources are identical or whether they are closely related alleles. In the absence of any recognized differences between their expression, however, it seems justifiable to apply the term pink-eye to the character in the newly found mouse as well as to the previously recognized form.

The existence of the pink-eye gene both in the population of *artemisiae* in Montana and either in the *sonoriensis* or *rubidus* population in California could have resulted either from two independent mutations at the same locus in the chromosome or from the transmission of the gene through the populations of

Peromyscus following a single mutation at this locus. The trapping points where the mice having these genes were obtained are separated by 600 to 900 miles. The probability for the transmission of a rare gene to the two localities in question following a single mutation is very low. The most plausible interpretation of the observations therefore seems to be that two independent mutations have occurred at the pink-eye locus.

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